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Effects of Seasonal Variations, Thyroid and Steroid Hormones, and Carp Pituitary Extract on the Artificial Production of Channel Catfish *Ictalurus punctatus* × Blue Catfish *I. furcatus* Hybrids

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Abstract.—The primary objective was to compare thyroid hormones and levels of carp pituitary extract for the artificial production of female *Ictalurus punctatus* × male *I. furcatus* hybrid catfish. The effects of different carp pituitary extract dosage rates (5, 6, 9, and 10 mg/kg), carp pituitary extract (6 and 10 mg/kg) supplemented with the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), or pregnenolone (DHP) were determined for inducing ovulation of female channel catfish, fertilization of channel catfish eggs with male blue catfish sperm, and hatching rate of these embryos. Hormone treatments thyroxine and triiodothyronine with carp pituitary extract, carp pituitary extract alone, and pregnenolone with carp pituitary extract used to artificially produce hybrid catfish were not different in terms of ovulation rates, eggs/kg, fry/kg body weight of female channel catfish, fertilization rates, or hatching rates ($P > 0.05$). These findings suggest that lower amounts of carp pituitary extract may be used to induce spawn of female channel catfish for production of channel-blue catfish hybrids and the addition of thyroid and steroid hormones is ineffective at the rates used in this study.

Hybrid channel-blue catfish (female *Ictalurus punctatus* × male *I. furcatus*) could have a significant impact on the catfish industry. Giudice (1966) first reported heterosis of hybrid channel-blue catfish, and their potential for aquaculture. The channel-blue hybrid has outperformed all other commercially raised catfish (Yant et al. 1976; Dunham and Smitherman 1987; Argue et al., in press). This is the only hybrid among 28 interspecific hybrids evaluated within the family *Ictaluridae* to exhibit overdominance for traits desirable for intensive aquaculture (Dupree et al. 1966; Dunham et al. 1982; Dunham and Smitherman 1983).

Channel catfish are sequential spawners. Females lay eggs over a period of about 6 h after a prolonged courtship dance with the male (Clemens and Sneed 1957). Male catfish clean and prepare a nest site, preferably in a covered or protected area that is easily defensible. Females are attracted to males by pheromones, which trigger courtship dances and other biophysical cues that induce ovulation and final oocyte maturation (FOM) (Timms and Kleerekoper 1972; Ostroumouv 1997). After spawning, males guard the egg mass while continuously fanning the mass with their tail providing a flow of fresh water over the eggs.

Overcoming natural fish reproductive cues using exogenous hormones was first reported in 1935 (von Ihering 1935). Luteinizing hormone releasing hormone (LHRH) and its analogs (Busch and Steeby 1990), human or equine chorionic gonadotropin (HCG or ECG) (Sneed and Clemens 1960), ovaprim (Goudie et al. 1992), amphibian pituitary (Salami et al. 1996), carp pituitary extract (CPE), and pituitaries from channel catfish, flathead catfish *Pylodictis olivaris,* buffalo fish *Ictiobus cyprinellus,* and alligator gar *Lepisosteus spatula* (Sneed and Clemens 1968) have proven effective for inducing ovulation of catfish.

LHRH, HCG, CPE, and several other non-piscine pituitary gland extracts are effective ovulation inducing agents in *Heterobranchus longifilis* (Nadukwe et al. 1993), *Clarias gariepinus* (Huisman 1986), *C. batrachus* (Manickam and Joy 1989), *C. mac-*

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rocephalus (Tan et al. 1996), *Siluris glanis* (Kouril et al. 1996), *Heteropneustes fossilis* (Alok et al. 1993), and the hybrid production of *C. macrocephalus* × *Pangasius sutchii* (Na-Nakorn et al. 1993), *H. fossilis* × *C. batrachus* (Padhi et al. 1995), *C. gariepinus* × *H. longifilis* (Hecht and Lublinkhof 1985), and *C. gariepinus* × *C. batrachus* (Rahman et al. 1995). CPE is the most predictable compound for the timing of ovulation in channel catfish (Liu et al. 1997).

The precise mechanisms whereby CPE exerts gonadotropic activity has not been clearly elucidated considering the very complex hormonal control and feedbacks in teleost oogenesis and ovarian development (Grizzle 1985; Redding and Patino 1993; Patino 1995, 1997). A recent expressed sequence tag analysis indicated that CPE may exert its effects both directly on the ovarian thecal and granulosal tissues and indirectly by inducing gonadotropin gene expression, especially that of GtH II β-subunit with almost 10-fold induction (Karsi et al., in press).

Two maturation inducing hormones (MIHs) have been isolated that promote cyclin B synthesis in teleost oocytes: 17α-20β-dihydroxy-4-pregnen-3-one (DHP) (Nagahama and Adachi 1985) and 17α-20β, 21-trihydroxy-4-pregnen-3-one (20β-S) (Trant et al. 1986). Mechanically stripped gametes in channel catfish can be underripe, ripe, and over-ripe. Sturgeon eggs not having undergone FOM dissolve in hypotonic solutions or in water (Dettlaff and Vassetzky 1988). This is symptomatic of unfertilized catfish eggs. Immature or dormant eggs may be released during induced ovulation and stripping. As such, they would be ovulated or stripped in a 4n state in the dormant phase of meiosis I. Alternatively, CPE may be overriding this system, and eggs may be expelled in an overripe condition (Liu et al. 1997). Efforts to bridge these gaps by injection of the pregnenolone ester DHP has not been assayed in channel catfish.

Thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) play a significant role in fish embryogenesis, morphogenesis, larval growth, fry survival, and juvenile development (Dales and Hoar 1954; Woodhead 1966; Lam 1980, 1994; Fagerlund et al. 1984; Lam and Sharma 1985; Lam et al. 1985; Inui and Miwa 1985; Miwa and Inui 1987; Brown et al. 1988, 1989). T_3 is the active form of thyroid hormone, and it alone can increase hatching rates of fish spawned under artificial conditions (Tachihara et al. 1997). T_4 is produced by the thyroid then converted to its active anabolic form, T_3, in the liver by the enzyme 5′-monodeiodoaminase (Higgs et al. 1974; Eales and Brown 1993; Lam 1994; Brown 1994). Fish embryos are not capable of producing thyroid hormones and are dependent upon thyroid hormone reserves in eggs. Fully mature oocytes depend upon successful translation of endocrine signals which regulate maternally derived loading of thyroid hormone (Brown et al. 1989; King et al. 1997). Concentration of T_4 in eggs has been shown to correlate with increased levels of T_4 in the serum of some freshwater teleosts (Tagawa et al. 1990; Lam 1994). Eggs have high concentrations of thyroid hormone prior to embryo cell differentiation and proliferation (Tagawa et al. 1990). As fish larvae develop, concentrations of these thyroid hormones in the eggs decrease (Lam 1994).

Thyroid hormones were applied to gravid females, fertilized eggs, and recently hatched larvae in efforts to improve seed production, increase hatching rates and fry survival (Baker-Cohen 1961; Lam 1980; Inui and Miwa 1985; Brown et al. 1988, 1989; Tachihara et al. 1997). Direct immersion of eggs with T_3 and T_4 (Lam 1980; Lam et al. 1985), oral administration (Higgs et al. 1976), or females injected intramuscularly with T_3 prior to ovulation (Brown et al. 1988, 1989; Tachihara et al. 1997) have been used to expose eggs to these hormones. Although T_3 injected into brown trout *Salmo trutta* had adverse effects on hatching rates and fry survival (Mylonas et

al. 1994), improved fertilization and hatching rates were generally reported after administration of T_3 or T_4 into females in teleosts at 10–100 mg/kg female body weight (Ayson and Lam 1993; Lam 1994) and in stellate sturgeon *Acipenser stellatus* (Dettlaff and Davydova 1979).

The objectives of the present research were: 1) to determine the effects of T_3, T_4, and DHP in combination with CPE on ovulation and fertilization of channel catfish females, and hatching rate of hybrid channel-blue catfish embryos, and 2) to determine the effects of varying amounts of CPE. In addition, to gain insight into effects of seasonal variations, rates of ovulation, fertilization, and hatching were analyzed based on date of spawning.

Materials and Methods

Experimental Fish

Female and male channel and blue catfish maintained at the Fish Genetics Research Unit, Alabama Agricultural Experimental Station, Auburn University were used in this experiment. Brood stock was fed 32% protein floating catfish feed from June 1996 until January 1997, then fed 48% protein catfish feed two times per week until spawning season. This diet was supplemented with raw chicken liver once per week from January until May. Feeding rate was 3% of total biomass of brood fish stocked per pond.

A total of 165 female channel catfish were spawned in this study. Several genotypes were used: Kansas (K), Marion (M), and Auburn (A) strains, and lines derived from their crosses. Male blue catfish used to make hybrids were Commercial, D & B, Auburn-Commercial, and Tombigbee strains (Dunham and Smitherman 1987). Female body weights ranged from 1.0 to 4.5 kg, and male blue catfish body weights ranged from 2.0 to 8.0 kg. Fish were spawned once a week for 11 wk.

Preparation of Pituitary Solutions

Whole carp pituitary glands (Stollers, Iowa) were weighed and ground in plastic 15-mL test tubes, then dissolved in saline solution using a Tissue Tearor™ (Biospec Products). After centrifugation (2 min, 3,000 r.p.m. in an SS-34 rotor, 20 C), precipitate was discarded and the prepared supernatant dilutions of 1 mg/mL and 4 mg/mL were set aside and refrigerated until used 12 h later for priming injections, and 24 h later for resolving injections.

Hormone Injections

Females were given priming injections of CPE followed 12 h later by resolving injections. The regimes tested were (mg CPE/kg female body weight, priming: resolving injections) 1:4 (N = 16), 2:4 (N = 28), 1:8 (N = 18), and 2:8 (N = 49).

Another set of experiments tested the effects of thyroxine (T_4, sodium salt, Sigma) and 3,5,3′-triiodothyronine (T_3, sodium salt, Sigma). T_3 and T_4 were administered by IP injection at 20 mg/kg body weight (Brown et al. 1988; Tachihara et al. 1997) during CPE priming injections of 2 mg/kg female body weight. A resolving dose of either 4 mg/kg (N = 10 and N = 12 for T_3 and T_4, respectively) or 8 mg/kg (N = 8 and N = 13 for T_3 and T_4, respectively) of CPE then followed 12 h later. T_3 and T_4 were weighed and then dissolved in 1 mL of dimethylsulfoxide (DMSO, Sigma) and then diluted in 200 mL of CPE solution. The final concentration of DMSO was kept at 0.5% (v/v).

A third treatment tested the effects of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP, Sigma) as a CPE supplement. DHP was administered at 30μg/kg body weight (N = 10) during the CPE priming dose. This concentration should be adequate since DHP stimulated 100% germinal vesicle breakdown of salmon oocytes at concentrations 2.5–5.0 ng/mL (Goetz and Hennessy 1984). Since pregnenolone is not water soluble, it was dissolved in 1 mL of 30% ethanol, then added to a CPE priming injection solution administered at a rate of 2 mg/kg body weight. The final alcohol concentration was 0.2%. The resolving dose was 4 mg CPE/kg female body weight.

Administration of Hormone Injections

Two days before the anticipated spawning day, females were seined from ponds and transported to an indoor holding facility. They were held together in a single, fiberglass holding tank supplied with continuous flow-through water maintained at 28 C. Individual females were randomly chosen, weighed to the nearest 0.01 kg, then administered the appropriate hormone and dosage. An 18-gauge needle was used to administer all injections. Females that received similar injections were placed together in holding tanks randomly designated by treatment.

Collection and Fertilization of Gametes

Twenty-four hours after the second injection, females that expressed eggs when handled were removed from holding tanks and anesthetized in 250 mg/L tricaine methane sulfonate (MS-222). Females were sequentially monitored for ovulation before they were anesthetized until all experimental females had been examined for ovulation, stripped, then placed in a holding tank for restocking in outdoor ponds. Females that did not express eggs were returned then rechecked during another rotation. Stripping of gametes ceased when all females had been stripped or attempts to strip them had been made.

The abdomen of ovulating females were dried with a clean towel, then eggs were dry stripped into plastic bowls lubricated with vegetable shortening and volumetrically enumerated. Strain and weight of each female was recorded. Eggs were held in Hank's balanced salt solution (HBS) until fertilization. The pH 6.9 solution was prepared by dissolving the following in 20 L of reverse-osmosis water: 160 g NaCl; 8 g KCl; 2.8 g $CaCl_2$; 4.0 g $MgSO_4 \cdot 7H_2O$; 2.4 g $Na_2HPO_4 \cdot 7H_2O$; 1.2 g KH_2PO_4; 7.0 g $NaHCO_3$; and 20 g of glucose. Eggs were fertilized within 15 min of stripping.

Male blue catfish were sacrificed and testes were removed, then weighed. Sperm was removed from the testes by macerating them into HBS (Bart 1994). A total of 0.5 g of testes was used per 100 mL of eggs. Testes from two or more males were macerated together in HBS in the event one male had unviable sperm. Four grams of testes were held in 20 mL of HBS. The HBS-sperm solution was mixed with individual egg masses followed by the addition of approximately 1 L of water. Activated gametes were allowed to sit undisturbed for 2 min, then an additional 5 L of water was added. Every 15 min water was decanted and replaced with fresh water. After egg masses developed an adhesive matrix and had water hardened for 1 h, they were placed in wire mesh baskets suspended in paddle wheel incubation troughs. Egg masses were treated with 100 ppm formalin 3 times per day until 1 to 2 d before hatch. All water used originated from surface runoff water sources and was held in a reservoir. Water temperature was maintained at 28 C. Total hardness and alkalinity of the holding water was 34 and 28 ppm, respectively.

Forty-eight hours after fertilization, three samples were randomly chosen from incubating egg masses to enumerate eggs. Fertilization rates were determined at this time. Eggs were considered fertilized when embryo neural ridge development and tail bud movement was evident as viewed in a glass petri dish held above a 75-watt bulb. Egg mortality was monitored by volumetrically enumerating individual egg masses at 48, 72, and 96 h post-fertilization. Upon hatching, fry were siphoned from the paddle wheel troughs then volumetrically enumerated. Hatching rates of individual females were calculated by dividing the total amount of fry hatched per treatment by the total number of eggs stripped or collected from individual females per treatment.

Statistical Analysis

All data was analyzed using SAS 6.12 (SAS Institute, Inc., Cary, North Carolina). A completely randomized factorial design

TABLE 1. *Percent ovulation of female channel catfish* Ictalurus punctatus *using different ovulating agents supplemented with various hormones (DHP = 17α, 20β-dihydroxy-4-pregnen-3-one, 30 μg/kg; T_4 = thyroxine, 20 mg/kg; T_3 = triidothyronine, 20 mg/kg; CPE = carp pituitary extract, priming:resolving injections, mg/kg. Means for treatments were not significantly different (P > 0.05).*

Hormone treatment	*N*	Percent ovulation
CPE		
1:4	16	62
2:4	28	76
1:8	18	75
2:8	49	87
Thyroid hormones with CPE		
2:4T_3	10	100
2:4T_4	8	75
2:8T_3	12	83
2:8T_4	14	93
DHP with CPE	10	75

was used for all experiments. Ovulation, fertilization and hatching percentage means were transformed using the arcsine of their square root before statistical analysis (Zar 1984). A mixed model ANOVA was used in all analyses since the factorial design of the experiment had both fixed and random effects (Zar 1984).

Results

Ovulation

There were no differences in percent ovulation among the different hormone treatments used to induce female channel catfish to spawn over the course of the spawning season ($P = 0.15$, Table 1). Combination treatments of two CPE priming doses (1 and 2 mg/kg body weight) and two resolving doses (4 and 8 mg/kg body weight) were not different in terms of ovulation rates (62%, 76%, 75%, 87%; 1:4, 1:8, 2:4, 2:8, respectively).

No significant effects were observed with treatments of CPE at two doses (2:4 and 2:8) when supplemented with T_3 (100% and 83%, respectively) or with T_4 (75% and 93%) (Table 1). Similar results were obtained when supplemented with DHP (75%). Thus, carp pituitary alone was enough for induction of ovulation. Additional treatment with thyroid hormones or pregnen hormones did not improve ovulation rates (Table 1).

Eggs Stripped and Fry Produced from Artificially Spawned Females

Variable levels of CPE did not have significant effects on numbers of eggs produced from unit weight of channel catfish females. Hormone treatments with CPE at 5 mg/kg (4,591 eggs/kg), 6 mg/kg (3,908 eggs/kg), 9 mg/kg (4,483 eggs/kg), or 10 mg/kg (5,708 eggs/kg) were not statistically different.

Eggs produced per kg body weight of female were not different when supplemented with DHP (7,278 eggs/kg), 6 mg/kg CPE and T_3 or T_4 (4,725 and 6,046 eggs/kg, respectively), or 10 mg/kg CPE and T_3 or T_4 (4,817 and 5,692 eggs/kg, respectively) ($P > 0.05$, Table 2). Carp pituitary extract supplemented with DHP was not different from CPE only for eggs (7,898 and 7,279, respectively, $P = 0.87$) and fry (1,060 and 1,228, respectively, $P = 0.98$) produced per kg of female (Table 2).

Fertilization and Hatching Rate

There were no differences observed in fertilization or hatch rates of manually stripped channel catfish eggs fertilized with male blue catfish sperm when CPE was supplemented with the thyroid hormones T_3, T_4, or DHP as compared to treatment with CPE alone (Table 3). When CPE 2:8 and 2:4 CPE-DHP treatments were compared (spawning week 8), there was no difference between fertilization rates (79% and 80%, respectively, $P = 0.20$) or hatching rates (14% and 16%, respectively, $P = 0.85$) (Table 3). There were no differences between fertilization ($P = 0.17$), eggs/kg female ($P > 0.05$), hybrid embryo hatching rate ($P = 0.88$), or fry/kg female ($P = 0.13$) when the different CPE treatments were compared. No differences were found be-

TABLE 2. *Number of eggs stripped/kg female channel catfish* Ictalurus punctatus *injected with carp pituitary extract (CPE), CPE supplemented with T_3 (triiodothyronine) or T_4 (thyroxine), and CPE supplemented with DHP (17α,20β-dihydroxy-4-pregnen-3-one).Numbers represent mg hormone/kg of priming injections followed 12 h later by resolving injections. Mean number of eggs stripped per kilogram of female and fry produced per kilogram of female are not significantly different (P = 0.80 and P = 0.30, respectively) among injection protocols.*

Treatment	N	Number of eggs per kg female ± (SEM)	Number of fry per kg female ± (SEM)
CPE			
1:4	9	4591 ± 916	459 ± 88
1:8	11	3908 ± 1736	178 ± 119
2:4	29	4483 ± 463	1053 ± 219
2:8	38	5708 ± 459	723 ± 133
CPE/T_3			
2:4T_3	10	4725 ± 390	601 ± 173
2:8T_3	10	4817 ± 519	790 ± 228
CPE/T_4			
2:4T_4	6	6046 ± 838	801 ± 309
2:8T_4	13	5692 ± 593	1501 ± 517
CPE/DHP			
2:4 DHP	6	7278 ± 589	1228 ± 326

tween hatching rates of artificially produced hybrid catfish when different CPE treatments were compared over the entire spawning season ($P = 0.13$).

Variations of Spawning Season on Fertilization and Hatching Rates

Spawning season variations appeared to have a major effect on fertilization and hatching rates (Fig. 1). When fish treated with CPE were analyzed based on spawning dates, a clear pattern was observed with the period between June 5 to June 26 being the most optimal for high fertilization and hatching. The fertilization rates on June 5 were over 80%, doubling those on other dates early during the spawning season. Similarly, the hatching rates were highest on June 18 and June 26 with over 30% of hatching for the hybrid. The hatching rates were generally low early in the spawning season (Fig. 1) and late in the season after

TABLE 3. *Comparison of mean fertilization and hatching rates of hybrid catfish embryos produced from female channel catfish* Ictalurus punctatus *mated with male blue catfish* I. furcatus *induced to spawn using different rates of carp pituitary extract (CPE) supplemented with T_4 or T_3. Fertilization and hatching rate means are not significantly different (P > 0.05). DHP experiment was conducted in a different date in the spawning season.*

Treatment	N	Mean % fertilization ± SE	Mean % hatching rate ± SEM
CPE:			
2:4	13	63 ± 9	24 ± 6
2:8	12	70 ± 9	17 ± 4
CPE + thyroid hormones:			
2:4T_3	10	73 ± 10	12 ± 3
2:4T_4	6	77 ± 6	12 ± 4
2:8T_3	10	71 ± 9	15 ± 4
2:8T_4	13	72 ± 8	23 ± 7
CPE	9	80 ± 5	14 ± 2
CPE + DHP	6	81 ± 8	16 ± 3

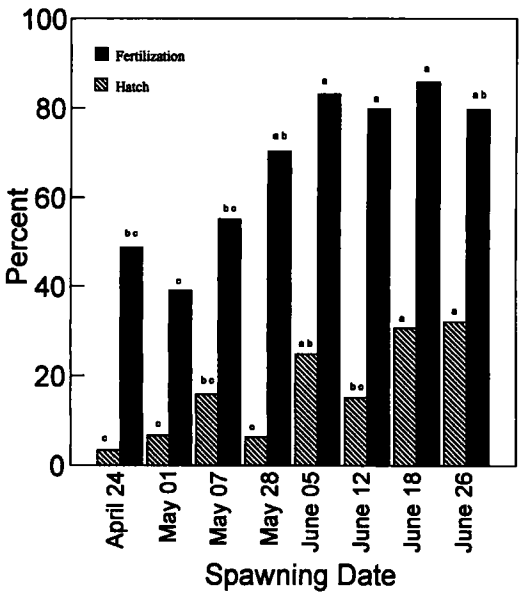

FIGURE 1. *Hatching and fertilization rates of channel catfish* Ictalurus punctatus *eggs fertilized by male blue catfish* I. furcatus. *Means (bars) ± SEM with different letters are significantly different (Tukey's t-test, P < 0.05), while those sharing the same letter are not significantly different. Females were induced to spawn using carp pituitary extract, then manually stripped. Eggs were artificially fertilized using male blue catfish sperm.*

July (data not shown). Means for fertilization and hatching rates were statistically different later in the spawning season as compared to those early in the season (Fig. 1, $P < 0.05$).

Discussion

Environmental variables regulating channel catfish reproductive physiology remain the primary controlling factors in egg quality. Egg quality and hybrid fry production are secondarily influenced by spawning techniques and brood stock management practices. This research demonstrated that seasonal variations had the significant effects on fertilization and hatching of channel catfish eggs from females treated with carp pituitary extract (CPE). Compounded into the seasonal variations are mainly temperature and photoperiod. Further analysis separating the effects of temperature from those of photoperiod is needed to assess their relative contributions. Several weeks in June appeared to be optimal for artificial spawning in channel catfish. Although temperature may change from year to year, it is reasonable to assume that mid-spawning season should be the period for artificial spawning and production of hybrid catfish. This is clearly demonstrated in this research and from our previous unpublished observations.

Levels of CPE, and supplements with T_3, T_4, or DHP did not have a significant effect on ovulation, egg production, fertilization, and hatching. Several explanations may account for these results. With ovulation, CPE alone is enough to induce spawning, thus effects of additional treatments may have been masked by the major effect of CPE, especially considering large variations. In addition, there could be interactions between the effects of CPE and those of thyroid hormones or steroid hormones. IP injection of thyroid hormones may not be an appropriate method of application for the compound. Other studies have found bath or dip treatments to be effective methods of hormone application (Piferrer et al. 1993; Al-Ablani 1997).

Overriding reproductive cues using exogenous hormones to induce ovulation remains the only sure control researchers and producers have over the reproductive physiology of channel catfish. Females can be fed high quality rations and can be consistently induced to ovulate using different compounds; however, this does not ensure egg quality or consistent levels of hybrid fry production. The use of additional thyroid hormones or DHP did not increase fry production in the present research. However, smaller quantities of CPE than currently widely used doses can be used to artificially produce hybrid catfish during the optimal spawning period than previously reported (6 mg/kg female body weight versus 10 mg/kg). Once again, it is highly important to conduct artificial spawning in mid-spawning season when the compounding factors of temperature and photoperiod may be optimal, in spite of current lack of information on their relative contributions.

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