

**Controlled Spawning of White Catfish, *Ictalurus catus*, and Brown Bullhead Catfish, *Ameiurus nebulosus*, Using Carp Pituitary Extract and LHRHa**

by

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## Abstract

If successful artificial spawning techniques can be developed, white catfish, *Ictalurus catus*, and brown bullhead, *Ameiurus nebulosus*, may have the potential to become model organisms for accelerated catfish genetics research due to their putative shorter maturation times and smaller handling size. Carp pituitary extract (CPE) and luteinizing-hormone releasing-hormone analogue (LHRHa) are two hormones used for successful spawning in commercially important channel catfish, *I. punctatus*, and blue catfish, *I. furcatus*. CPE injection, liquid LHRHa injection, LHRHa ethylene vinyl acetate copolymer (EVAC) implant, and LHRHa cellulose acetate (CA) implants were used for spawning trials in white catfish, while CPE injections and liquid LHRHa injections were used for spawning trials in brown bullheads. In 2010, LHRHa EVAC implants in domestic white catfish resulted in near-simultaneous ovulation for 100% of the females after 72 hours (1944°-hours). In 2011, LHRHa cellulose acetate implants yielded no ovulation for wild white catfish, while CPE injections resulted in 28.6% ovulation. In 2012 wild white catfish injected with liquid LHRHa did not ovulate. Water flow appears to play a key role in successful spawning as CPE injected wild-acclimated and 1- year- old domestic white catfish, both for which water flow was interrupted, were the only white catfish treatments able to spawn (50% ovulation;1550°-hours and 75% ovulation;1211°-hours, respectively). Brown bullheads did not ovulate in response to any LHRHa treatments across years. In 2012, all wild-acclimated brown bullheads given a CPE treatment with interrupted water flow were able to ovulate (<2100°-hours). All other brown bullhead treatments with CPE and liquid LHRHa injections and with continuous water flow did not

ovulate. Domesticated white catfish were capable of spawning at 1 year of age. This early maturation time makes them a candidate for accelerated catfish genetics and xenogenesis research.

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## List of Abbreviations

CPE	Carp pituitary extract
LHRHa	Luteinizing-hormone releasing-hormone analogue
EVAC	Ethylene vinyl acetate copolymer
MS-222	Tricaine methanesulfonate
°-hours	Degree-hours (temperature °C · number of hours)
Mg/L	Milligrams per liter
Mg/kg	Milligrams per kilogram
ppm	Parts per million
KMnO <sub>4</sub>	Potassium permanganate
IP	Intraperitoneal
IM	Intramuscular



## List of Fishes

Common name:	Scientific name:
Blue catfish	<i>Ictalurus furcatus</i>
Brown bullhead	<i>Ameiurus nebulosus</i>
Channel catfish	<i>Ictalurus punctatus</i>
Flathead catfish	<i>Pylodictus olivaris</i>
White catfish	<i>Ictalurus catus</i>

## Introduction

White catfish, *Ictalurus catus*, and brown bullhead, *Ameiurus nebulosus*, may be good model species for transgenic, xenogenic, and other genetic research for later maturing and larger ictalurids such as channel catfish, *Ictalurus punctatus*, and blue catfish, *I. furcatus*. White catfish and brown bullheads are believed to reach sexual maturity at one year of age compared to two and six years of age for channel catfish and blue catfish, respectively (Dunham and Smitherman 1981; 1984), which would allow acceleration of many types of research. However, this age at first maturity has never been verified, and currently there are no published data concerning successful spawning techniques for white catfish and brown bullhead.

### ***White catfish***

The taxonomy of the white catfish has been altered several times periodically placing them in one of two genera, *Ameiurus*, or “bullheads” with an “unforked caudal fin,” and *Ictalurus*, or “fish cats” known for deeply forked caudal fins (Etnier and Starnes 2001). White catfish can be characterized by large heads which are wider than their bodies. White catfish are typically blue/gray dorso-laterally and white in color on the ventral side (Etnier and Starnes 2001). However, some populations of white catfish from the Southeastern U.S. can be very dark gray or almost black dorso-laterally (M. Fobes, unpublished observation). The ventral barbels (chin barbels) are white, as opposed to some of the *Ameiurus* species, black bullhead, *A. melas*, and brown bullhead, which both have dark colored barbels (Etnier and Starnes 2001). However two *Ameiurus* species also have light colored chin barbels, yellow bullhead, *A. natalis*, and the flat bullhead, *A. platycephalus*. Bullheads with light chin barbels can still be discriminated from the

white catfish based on morphological differences of the head as well as body coloration. White catfish have a moderately forked caudal fin, unlike many of the bullhead species. The head morphology alone should suffice to differentiate the white catfish from species in the genus *Ictalurus*, whose heads are not as large relative to their body thickness. The combination of coloration, head morphology, and ventral barbels can help to distinguish it from other species found in both genera, regardless of any possible taxonomic changes in the future.

In terms of age, studies have shown maturity beginning in white catfish at 2.9 to 4.8 years old in river environments and 2.3 to 3.5 years in lake environments in the northeastern U.S. (Caromile 1994) in contrast to the speculation of Dunham and Smitherman (1981; 1984) that they can become mature in 1 year. In terms of body length, onset of sexual maturity in white catfish has been observed to begin as early as 152 mm fork-length (FL) in populations in northern California (Borgeson and McCammon 1967) and up to 230 mm total length (TL) in a New Jersey population (Keller 2010).

According to length-weight relationship data on white catfish in the Santee-Cooper Reservoir system from Stevens (1959), fish of approximately 160 mm and 230 mm had average weights of 0.06 kg and 0.14 kg, respectively. Studies on the culture of white catfish in brackish water ponds show their growth to be as rapid as 0.45 kg after 9.5 months of being on a commercial feeding regimen (Perry and Avault 1968) and 0.32 kg after 222 days (Perry and Avault 1969). Both studies started with fingerlings approximately 0.023 kg in weight. Growth data from Perry and Avault (1969) show that white catfish outgrew both channel and blue catfish for the first 4 months of culture. In less than 1 year, these cultured white catfish of 0.45 kg and 0.32 kg, according to the length-weight relationship found by Stevens (1959), would be at lengths between 304mm – 330mm and 228mm – 251mm, respectively. These lengths are within, or well

beyond, the previously observed lengths at maturity for white catfish populations (Borgeson and McCammon 1967; Keller 2010). The fish from Perry and Avault (1968; 1969) may have grown even faster if water parameters were similar to more freshwater settings, instead of brackish. However, Perry and Avault (1968; 1969) made no mention of their white catfish exhibiting any sexual maturity, and their results on reproduction were inconclusive for all 3 species, possibly due to high salinities.

White catfish spawning began in late May in the Sacramento-San Joaquin Delta, California when water temperatures are 19 - 21°C (Borgeson and McCammon 1967; Wang 2010). Spawning began later, and at higher temperatures, from June to early July in more northerly latitudes such as in a Delaware river estuary when water temperatures reached 23-25°C (Keller 2010), and in June to July in a Hudson river estuary in Albany, NY (Jordan et al. 2004).

### ***Brown bullhead***

Brown bullheads can be characterized by dark brown or gray coloration dorsally and mottled sides (Etnier and Starnes 2001). All of their barbels are dark, as are the barbels of the black bullhead, but the brown bullhead has a more elongated body unlike the short and deep bodied black bullhead.

Brown bullheads have been of interest to researchers due to their ecological interactions with their environment. They can survive in very low oxygen levels (Cooper and Washburn 1949; Baumann et al. 2008), even as low as 0.2-0.3 ppm, while still showing a large rate of survival (Cooper and Washburn 1949). Their bottom feeding behavior puts them in direct contact with contaminants embedded in the sediment, thus, they have been used as bio-indicators of ecosystem health and contamination (Baumann et al. 1987; Ali et al. 1993; Baumann and

Harshbarger 1995, 1998; Leadly et al. 1999; Arcand-Hoy and Metcalfe 1999; Steyermark et al. 1999; Baumann et al. 2008). Because of their use as indicators, laboratory and tank studies to better understand physiological reactions of brown bullheads with contaminants will likely increase (Iwanowicz et al. 2006).

After excluding adults that were found to not be a part of a spawning pair, Blumer (1985) observed mature brown bullhead adult mean standard lengths (SL) to be ~230 mm for males and ~224 mm for females. Length-at-age records in a New York population showed fish at these sizes to be 2-3 years of age (Sinnott and Ringler 1987). As with the white catfish, it may be possible for brown bullheads in culture conditions to outgrow bullheads in wild environments and reach mature lengths earlier.

Brown bullhead spawning in northern California populations began as early as April and ending as late as August (Wang 2010). Favorable temperatures for these California populations allow spawning to begin when water temperatures reach 21°C (Wang 2010), while Michigan populations began spawning when temperatures were lower (Blumer 1985).

### ***Spawning***

Three options for spawning catfish exist including natural pond spawning, pen spawning, and aquarium or tank spawning (Bates & Tiersch 1998). While the white catfish and brown bullhead are capable of spawning naturally in fish culture ponds on their own (M. Fobes, personal observation), controlled artificial spawning, including in-vitro fertilization techniques, is needed for genetic manipulation (Dunham 1993).

Male ictalurid catfish have villiform testes preventing collection of milt from manually stripping the males, although minute quantities of milt have been collected from blue catfish

using this procedure (Dunham 1993). Thus, males must be sacrificed and their testes manually removed and pulverized to extract the gametes for manual fertilization.

For temporally controlled spawning in pens, tanks and aquariums, a variety of hormones have been tested on channel catfish. Carp pituitary extract (CPE) and luteinizing hormone-releasing hormone analogue (LHRHa) are two well-known hormones for controlling ovulation in channel catfish. Carp pituitary extract had begun to be used at rates of 11 mg/kg female body weight divided into an initial preparatory dose of 2 mg/kg and a resolving dose of 9 mg/kg administered 12 hours later, with initial results showing 90-100% ovulation success (Bidwell et al. 1985). However, due to over-ripening of eggs the dosage was scaled back to an 8 mg/kg resolving dose (Dunham 1993). Experiments using LHRHa at 100 µg/kg female body weight showed spawning success comparable to human chorionic gonadotropin (HCG) and better than CPE and control groups (Busch and Steeby 1990).

Channel catfish females are always hormone induced in aquaria or in confined environments for release of eggs when males are not present (Bates and Tiersch 1998). Auburn University was the first to report that female channel catfish could be induced to ovulate without the presence of males (Dunham 1993, Bart and Dunham 1996, Bart et al. 1998, Dunham et al. 1998, 1999, Lambert et al. 1999). Data from several experiments show that ovulation of channel catfish females induced with LHRHa injectable (several hundred fish) or LHRHa implants (several thousand fish) routinely ranges from 60-100% with a mean close to 90% (Kristanto 2004, Hutson 2006, Ballenger 2007, Phelps et al. 2007, Kristanto et al. 2009, Su et al. 2012). In the case of the injections, a priming dose of 20-30 µg and a resolving dose of 100-150 µg/kg 12 hours later was used. Bates and Tiersch (1998) obtained a 58% ovulation rate of groups of

channel catfish females injected with LHRHa in a re-circulating system. In this case, a single injection of 100 µg/kg was utilized.

Chatakondi et al. (2011) used low dosages of LHRHa 20/40, 20/60 and 20/80 (priming dose/resolving dose) µg/kg, and at a 15 hour interval. Ovulation rates and fry/kg were poor for this experiment, 45.0-52.9% and 362-687 fry/kg, respectively. These poor results may be a result of the low doses used, the slightly excessive time period between injections and poor brood stock preparation.

If a priming and resolving dose are used, ovulation can be predicted within a few hours (Kristanto 2004, Hutson 2006, Ballenger 2007, Phelps et al. 2007, Kristanto et al. 2009) allowing this procedure to be used on a commercial scale. Time of ovulation is not predictable without hormone induction, and predictable times of ovulation are needed to facilitate various experiments.

To date, no published data exist for induced spawning and artificial fertilization in white catfish and brown bullhead species. If white catfish and brown bullheads are to be successfully used as model species, artificial spawning of these species needs to be reliable and their minimum age of sexual maturity verified. The objective was to develop a protocol for the hormone induced spawning of white catfish and brown bullheads in a research/experimental setting and determine if these fish can become sexually mature and ovulate at 1 year of age.

## Methods & Materials

The experiment was conducted at the Auburn University Fish Genetics Unit, E.W. Shell Research Center, of Auburn University, Alabama. White catfish brood stock were obtained from St. Johns River, Florida in April 2011, Santee-Cooper Reservoir, South Carolina, and from Farm Pond 11 (FP-11) at Auburn University in 2010 and 2011, which contained domestic white catfish originally from the Santee-Cooper system, South Carolina. Fish from these locations were obtained through electroshocking and trotlines. Brown bullhead catfish brood stock were obtained from local water bodies around Auburn, AL, including FP-11, during the spring of 2011. Each species was stocked in a 0.04-ha earthen pond. Upon arrival at the Fish Genetics Unit, all fish were acclimated to the pond water by several partial water changes prior to stocking. Each species was placed in their own pond. Forage populations of bluegill (*Lepomis macrochirus*) and green sunfish (*Lepomis cyanellus*) were established prior to stocking the white catfish and brown bullheads. After stocking, the fish were fed 9kg/ha per day with a commercial floating pelleted feed (32% protein). However, a feeding response was never observed from the catfish.

### ***2010 Season***

Five gravid, domesticated white catfish females were each implanted with a single 90 µg/kg LHRHa EVAC implant and placed individually in aquaria. This treatment is shown in Table 1. Average water temperature was 27°C. Criteria for gravid female selection, for both species, were a soft swollen abdomen and a somewhat reddened and enlarged genital papilla.



## *2011 Season*

Controlled spawning for white catfish and brown bullhead began about 3 - 4 weeks after stocking in the earthen ponds. Attempts to spawn white catfish took place from May 17<sup>th</sup> through June 21<sup>st</sup>. The criteria for selection of gravid of females were identical to criteria followed in 2010.

All injections were intraperitoneal (IP) using an 18 gauge hypodermic needle, except for one occasion when intramuscular (IM) injections were performed, as explained below. Hormone was allowed to warm to ambient temperature before injection. Females were injected and immediately placed back into their individually assigned tanks and observed for signs of any released eggs at regular intervals.

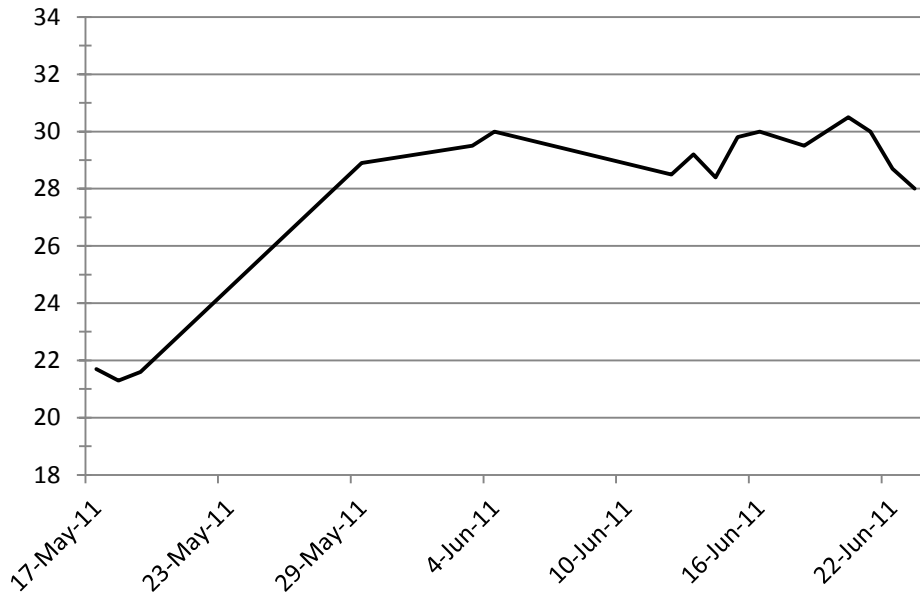
A total of 31 white catfish and 6 brown bullhead females were used for this season. Six different treatments were evaluated for white catfish, and 1 treatment for the brown bullheads (Table 1). Treatment 1 for wild white females was a single LHRHa cellulose acetate (CA) implant at a rate of 90 µg/kg body weight. Treatment 1 consisted of 4 females. Treatment 2 was injection of CPE at a rate of 10 mg/kg body weight, with a preparatory injection of 2 mg/kg body weight followed by a resolving injection at a rate of 8 mg/kg body weight 12 hours later, similar to the procedures for controlled spawning in channel catfish. These fish were kept individually in their own tanks. Treatment 2 consisted of 7 females. Females in treatment 3 were also injected with CPE at similar doses, but each female shared a tank with a single male. This treatment was used to determine if pheromonal communication could enhance ovulation. The male and female were kept separate using a divider to prevent the fish from laying egg masses before the eggs could be harvested. Treatment 3 consisted of 8 females. Females in treatment 4

shared tanks with a male just as treatment 3, but these fish were given a total of 5 injections in the following order: 2 mg CPE/kg preparatory, 8 mg CPE/kg resolving (12 hours later), 50 µg CA implant LHRHa/kg (84 hours later), 4 mg CPE/kg (36 hours later), and 4 mg CPE/kg (72 hours later). These fish were given more injections than standard, as well as the addition of a different hormone, as they did not respond to the initial injections, but still appeared gravid. Treatment 4 consisted of 4 females. Treatment 5 fish were similar to treatment 4 in that they also received more injections than standard, 4 total, except only CPE was used, and the rates differed as well: 2 mg CPE/kg preparatory, 8 mg CPE/kg resolving (12 hours later), 4 mg CPE/kg (72 hours later), and 8 mg CPE/kg (96 hours later). The initial 2 mg + 8 mg/kg injections were IM. The following injections were IP after individual fish showed red bumps at IM injection sites. No bumps were observed for IP injections. Treatment 5 consisted of 6 females. The sixth treatment for white catfish females for the 2011 season, were 2 females given the standard 2 mg + 8 mg CPE /kg procedure. These two females shared a tank with each other, with no males or dividers.

The only treatment for brown bullheads of this season was for 6 females given the standard 2 mg + 8 mg CPE /kg procedure. None of these fish shared tanks with males.

Treatments for 2011 are shown in Table 1. Fish in all treatments appeared to be in similar condition at the start of the experiment.

**Figure 1.** Mid-day water temperatures for 2011.



All females of this season were placed in 91cm x 32 cm x 61cm glass aquaria, with water levels averaging 25.5 cm, giving them ~141.5 L of capacity. These aquaria were on aquaria stands approximately 1.7 meters high from the floor. Males for fertilization were kept in tanks separate from the females. Flow-through water was used from a reservoir approximately half a kilometer away from the greenhouse. Flow rates averaged about 4.1 L per minute for aquaria. When necessary, water was warmed with a submersible heater. Water was cooled by opening a bottom drain in the reservoir to mix cool bottom water with warmer surface water. Water temperatures for 2011 are shown in Figure 1. Dissolved oxygen (DO) levels in the water were maintained between 4 and 5.5 mg/L with the use of air-stones. After 3 days post-resolving injection, fish which did not ovulate were hand stripped. Fish which received follow-up

injections (after their first 2mg/kg+8mg/kg treatment) were hand stripped about 24 hours after each follow up injection.

When testes were needed for fertilization, a minimum of two males were used. Testes were placed in dry plastic weigh boats containing 11ml of 0.9% saline solution per gram of testes. Mesentery and blood were manually removed from the testes using forceps and scalpel which were prewashed with freshwater first, then rinsed in saline. Testes were manually ground through a fine-mesh metal strainer into solution with the saline.

When females began releasing eggs, the females were removed from their tanks and placed in large buckets of fresh pond water containing equal parts of commercial tricaine methanesulfonate (MS-222) 100 ppm and sodium bicarbonate for a short time. When they were anesthetized, the females were immediately removed from the MS-222, rinsed with pond water and thoroughly dried with a towel. Eggs were then hand-stripped into metal pie pans which were pre-coated with a thin layer of vegetable shortening to prevent the eggs from sticking to the pan. For fertilization, sperm solution was spread uniformly across the egg mass. The egg mass was stirred and mixed to allow the sperm solution to cover all of the eggs. Fresh pond water was then added to the pie pan containing the egg/sperm mix, to activate the eggs and sperm. After fertilization, eggs were placed into commercial 10 L McDonald hatching jars. Water flow varied across McDonald jars; water flow was adjusted so that eggs were gently propelled no higher than half way up the jar. Egg treatments were given as a flow-through treatment, not static-water, of 1 - 1.5 ml (100-150 ppm) of formalin (39% formaldehyde) three times a day until hatching appeared imminent.

Regardless if the fish successfully spawned or not, all fish that were injected had their adipose fins clipped and stocked back to their original pond. At the appearance of any injury or possible onset of water-borne disease while in aquaria or holding tanks, fish received static-water treatments of approximately 2-3 ppm of potassium permanganate (KMnO<sub>4</sub>) (Darwish et al. 2009) for approximately 30 minutes. Water flow was restored after the KMnO<sub>4</sub> treatment.

Egg quality was determined based on the appearance of the egg mass. Eggs with yellow color, and healthy spherical shape, and gelatinous consistency when in a mass, were considered healthy. Egg masses deemed to be low quality were white, and were watery in consistency. Percent fertilization was estimated by visually inspecting the egg mass.

Catfish fry produced from the 2011 spawning season were reared in 109 cm x 31 cm x 53 cm fiberglass tanks water depth of 24 cm (138.7 L capacity) at roughly 100 fry per tank, with flow-through water. The fry were fed, depending on size, AquaMax ® DD-00, DD-01, and DD-03 (50% protein) until they reached approximately 7cm in length. Upon reaching this length they were stocked in 0.04-ha earthen ponds at 5000 fry/ha for grow out. These ponds were prepared with live forage of *Lepomis* and were assigned a feeding regimen similar to their parents to prepare them for future spawning.

### ***2012 spawning season***

Additional white catfish brood stock (88 individuals) were acquired from the St. Mary River, Georgia in early April 2012. Additional brown bullhead brood stock (39 individuals) were acquired from Lake Seminole, Georgia in late April 2012 as well. The same acclimation procedures were followed as in 2011, and each species was placed in their own 0.04-ha earthen

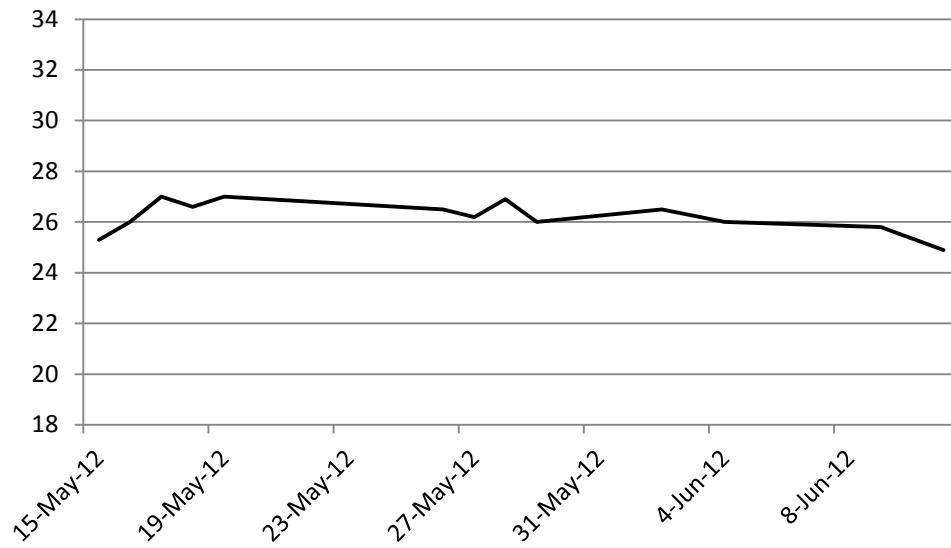
ponds at 2200 fish/ha (white) and 975 fish/ha (bullhead), not sharing the pond with any of the previously acquired fish. For the first two weeks, the new white catfish were fed a mix of diced chicken liver and commercial feed to encourage feeding activity and to adjust to the commercial feed. Minimal feeding activity was observed.

On May 15<sup>th</sup>, six white females acquired the previous year and 2 brown bullhead females acquired the previous year were IP injected with 100 µg LHRHa/kg body weight. White females were placed individually into the same aquaria used in the previous season. Brown bullhead females were also placed into the same aquaria used in the previous season, but each female was paired with a male with no divider. On May 25<sup>th</sup>, a factorial design experiment was implemented. For white catfish, the design was organized by genotype, hatchery history and by hormone. White catfish were divided into the following categories: wild, wild-acclimated, and domestic. Fish which had recently been acquired from natural settings, with only 4-5 weeks to acclimate to farm settings, were designated as wild. Wild fish acquired from the previous year (2011) which had approximately 1 year to acclimate to farm settings were designated as wild-acclimated. Fish that were the offspring of the 2011 season and were raised in the farm environment were designated as domestic. Within each group there were 2 hormone treatments: CPE and liquid LHRHa. This design resulted in 6 white catfish treatment groups. Three treatment groups were evaluated for the brown bullheads: a single wild group using LHRHa, and two wild-acclimated groups, one using LHRHa and the other using CPE. Treatments for 2012 are shown in Table 2. Regardless of hormone category, all treatments from both species received a preparatory dose of 2 mg CPE/kg body weight; hormone treatments differ in the resolving phase of injections. All CPE resolving doses were 8 mg CPE/kg body weight, and all LHRHa resolving doses were 100 µg LHRHa/kg body weight. Only IP injections were used.

Each treatment from both species was placed in a 484 L, non-transparent, fiberglass holding tank (27 cm of water x 59 cm x 304 cm) with a flow rate of approximately 6 liters per minute. All females from most treatments, from both species' experiments, were communally placed into a single tank with no dividers. The white, wild-acclimated-CPE treatment group was the only group which shared a tank with other fish. This treatment group shared a fiberglass tank with white males. The males and females were kept separate by a metal screen divider, with males downstream from the females to help prevent fertilization of released eggs. All fish were checked for eggs via hand stripping before termination of the treatment experiment.

Air stones were in all tanks to deliver air to maintain adequate DO levels. Dark colored, mesh bags were placed along the bottom of the tank to aid in spotting the light colored eggs. Mosquito netting was used as a tank cover for each treatment tank, to minimize sunlight. Water quality parameters in the tanks were: DO-8.32 ppm; hardness-26 ppm; alkalinity-32 ppm; carbon dioxide--~5 ppm; chlorides-16 ppm. After the experiment, on June 9<sup>th</sup>, two last gravid white females were found. These females were white wild-acclimated catfish who were given a 2 mg/kg + 8 mg/kg CPE injection. Based on the results of the factorial experiment performed on May 25<sup>th</sup>, these females were put in similar tanks with similar light conditions, but water flow was intentionally interrupted by being turned down to a trickle once 24 hours had passed after the resolving dose was administered, in hopes of increasing ovulation induction. All fish were hand stripped before termination of the hormone treatment.

Figure 2. Mid-day water temperatures for 2012



***Data Analysis***

Mean spawning percentage was calculated. Data were analyzed with Fisher's exact tests. Significance was evaluated at P=0.10 and P=0.05.



## Results

### *2010 spawning season*

Five domestic white catfish females were gravid in 2010. They were implanted with 90 µg/kg LHRHa EVAC implants. All 5 ovulated after 72 hours at 27° C (1944°-hours).

### *2011 spawning season*

All fish used in 2011 were wild. Four white catfish females were implanted with 90µg/kg female body weight using cellulose acetate implants, but none of these fish ovulated. The only successful treatment for white catfish was CPE injected females with 2 mg/kg female body weight for the priming dose and 8 mg/kg for the resolving dose that were in aquaria without other fish (Table 1). Two of 7 (28.6%) of these females ovulated. These females weighed 0.66 kg and 0.44 kg and ovulated, post-preparatory injection, at ~1334°-hours and ~1290°-hours respectively. Water temperature averaged 23° C for the 0.66 kg female. A total of 112.86 g of eggs were harvested, and the egg mass accounted for 17.1% of her total body weight prior to stripping. The water temperature for the 0.44 kg female's averaged 21.5° C, and a 72.3 g egg was produced, of which only 35.4% was deemed healthy by weight. The egg mass accounted for 16.4% of her total body weight before egg stripping. Females given the same regime that were in communal aquaria with males or other females did not give eggs. Brown bullheads given the priming dose of 2 mg/kg female body weight of CPE followed by a resolving dose of 8 mg/kg did not ovulate. White catfish females given multiple injections of CPE and LHRHa failed to

spawn. The CPE treatment without the presence of males yielded a higher ( $P=0.10$ ) ovulation rate than when males were absent.

Most white catfish males were communally held in fiberglass tanks for quick testes acquisition, and nearly half of them died from a *Flavobacterium columnare*, columnaris outbreak. The infections appeared related to lesions caused by fighting. Mortality was lower with males which were placed alone in divided holding tanks or with females.

Wild brown bullheads did not ovulate in 2011. Brown bullheads suffered the most mortality with nearly every individual taken out of their pond, male or female, dying after 2 or 3 days. Columnaris appeared to be the causative agent.

### ***2012 Spawning Season***

In 2012, wild-acclimated white catfish that were injected with LHRHa in individual aquaria, with LHRHa in communal tanks with other females, and with CPE in the presence of males did not ovulate (Table 2). One of 2 wild acclimated females that were injected together with CPE within a communal tank, for which water flow had been interrupted, ovulated. The fish (0.45 kg) that ovulated gave 79.1 g of eggs, volumetrically estimated to be (~3164 eggs), resulting in a relative fecundity of 7031 eggs/kg. Eggs were discovered 1550°-hours after preparatory injection. A 10g sample (400 eggs) was placed into a McDonald jar. Percent fertilization was visually estimated to be 95%, and hatching success was 62.9% (239 larvae from 380 eggs). No mortality in the post-hatch fry was observed. The female who did not ovulate was manually squeezed for eggs and gave none, despite appearing gravid. No additional injections were administered to the female.

Wild white catfish females, from the St. Mary River, injected with LHRHa or CPE in communal aquaria with other females did not ovulate. However, these wild fish naturally spawned in the earthen pond without spawning containers resulting in numerous white catfish fingerlings. Estimated water temperature at the time of spawning was 21° - 23° C.

One-year-old domestic white catfish injected with CPE that had their water flow interrupted had higher ( $P=0.05$ ) ovulation rate, 75%, than those injected with LHRHa with constant water flow, 0%. Time from preparatory dose to ovulation was about 47 hours, yielding a temperature degree-hour period of ~1211°-hours. Three of 4 CPE injected females gave eggs (the 4<sup>th</sup> female gave a single egg). The sizes of many of the eggs from all 3 of these fish were noticeably larger and smaller than the catfish eggs normally seen during the previous spawning seasons. Percent fertilization was visually estimated to be ~90%, and eggs were placed in McDonald jars. However, before any of the eggs matured into later stage embryos, all eggs died (losing yellow colored center and turned clear, eventually lysing). After manually stripping other treatments, only a single fish from the white-wild-acclimated, LHRH group was able to give a single over-ripe egg. All other females gave no eggs.

Wild and wild-acclimated brown bullheads injected with LHRHa did not ovulate. The only brown bullheads in the experiment to yield eggs were the 2 fish in the wild-acclimated in the CPE treatment with water flow interrupted. Their ovulation rate 100% was higher ( $P=0.05$ ) than the pooled LHRHa brown bullhead treatments, 0%.

Eggs were discovered 84h after preparatory injections. However, the females had already dropped numerous eggs throughout the bottom of the tank. Despite this, a total of 36.6 g of eggs were harvested. One female (0.35 kg) still had 26.1 g of eggs remaining, while the other female

(0.28 kg) gave 10.5 g of eggs in which only a minority of her total egg mass were of good quality. The larger brown bullhead's eggs exhibited a yellow color, gelatinous consistency, and were normal in appearance. Most of the smaller female's eggs were white and watery. Despite using three different males to fertilize the eggs, the brown bullhead eggs lost their yellow color approximately one hour or so after being placed into a McDonald jar, turned white and later dissolved. At the time of discovery of the eggs, approximately 2100°-hours had passed after preparatory injection, initial ovulation was missed and the eggs were over ripe.

Across 2011 and 2012, interrupted flow yielded greater ovulation ( $P < 0.0001$ ) than continuous flow. Domestic white catfish ovulated at greater rates than non-domestic white catfish ( $P < 0.03$ ) and wild white catfish ( $P = 0.03$ ) across 2011 and 2012. There was no difference ( $P = 0.14$ ) between ovulation of white catfish in covered tanks vs. aquaria or in ovulation rates between white catfish and brown bullheads ( $P = 0.4$ ). Ovulation rates between the 2011 and 2012 spawning seasons were not different ( $P = 0.19$ ).

## Discussion

Domestic white catfish responded more readily to induced-ovulation than wild white catfish, making them a better candidate as a genotype for application as a model species. EVAC LHRHa implants were highly effective, 100% spawning, for ovulating domestic white catfish females in 2010. The following year cellulose acetate LHRHa implants were ineffective, 0% spawning, for ovulating wild white catfish. In the case of channel catfish, EVAC and cellulose acetate implants are similar in efficacy (Dunham, unpublished) so the most likely explanation for the contrast of results is domestication, although the possibility that white catfish have a differential response to the implant delivery system cannot be totally discounted.

CPE is a powerful ovulating agent (Su et al. 2012) and wild white catfish sometimes ovulated when injected with CPE, but never responded to liquid LHRHa injections or LHRHa cellulose acetate implants. Wild-acclimated brown bullheads also responded to CPE, but not liquid LHRHa. These findings are in contrast to studies using channel catfish, which provided as good or better results using LHRHa than human chorionic gonadotropin or carp pituitary (Busch and Steeby 1990).

Female white catfish from 2011 were more likely to ovulate if individually isolated, and possible pheromonal communication with males or other females seemed to give no definite contribution to spawning. When custom dividers were used to allow both males and females to share aquaria, to possibly help ovulation through olfactory stimulation, white catfish females showed a behavioral reaction some hours later. Though data on the frequency of these behaviors were not recorded, white females were observed trying to swim over and/or jump over the

dividers to get to the males. Despite the aquaria having lids, some females succeeded in crossing the dividers and were found on the males' side of the tank. Regardless of females uniting with the males or not, females sharing tanks with males still did not release any eggs. It is counter intuitive that males would impede ovulatory response in this environment, especially since females made strong efforts to gain close proximity with the males, so another variable may have inhibited ovulation. This inhibitory effect was not observed during the 2012 season with communal domestic and communal wild-acclimated white catfish, all treated with CPE. Fish from the 2011 season may have been too stressed to mate due to high temperatures. However, no statistical difference among spawning rates between 2011 and 2012 was found. Despite any differences in temperature, no communal wild white catfish spawned in 2011 nor 2012, suggesting another variable was involved, possibly a domestication effect. However, this still does not explain why wild white females from 2011 were not able to spawn in communal settings, as opposed to wild white females not sharing tanks with other fish from the same year.

Water flow had been interrupted in some of the spawning tanks in 2012. Upon discovery of the disruption the water flow was restored. All ovulation of white catfish and brown bullheads occurred in these tanks in 2012. Fluctuating water flow may be a key to stimulating ovulation in these small catfishes, and needs further evaluation. This is also in contrast to results from studies spawning channel catfish, which found increased ovulation with an increase in water flow (Davis 1961, as cited by Brauhn 1971).

Ovulation occurred at 1944°-hours simultaneously for the 5 white catfish induced with LHRHa EVAC implants, and from 1211 to 1550°-hours for white catfish induced with CPE with a mean of 1146°-hours, and 2100°-hours or less for acclimated brown bullheads induced with CPE. Knowing this relationship, between species, hormone, and degree-hours, is critical for

artificial spawning and fertilization of white catfish and brown bullheads to ensure collection of high quality eggs. Phelps et al. (2011) found that a 3 hour delay in hand stripping an ovulating channel catfish can greatly reduce viability and hatch of the eggs collected. On more than one occasion in this study, females were found after ovulation was advanced, leading to potentially low egg quality. Either the females were not checked with enough frequency or they were able to hold ovulated eggs in their ovary without external release until egg quality was decreasing. If temperature degree hours for ovulation are known and have a small variance, the latter explanation can be addressed by stripping females on a schedule rather than upon release of eggs.

White catfish males were very aggressive and fought with each other when held communally. This resulted in wounds that became infected leading to death. For artificial fertilization protocols, the males will need to be isolated from each other.

A potential option for acquiring domestic white catfish would be to allow wild white catfish to spawn naturally in their ponds. If these wild adults produce fry on their own, as previously observed, the parents may be seined out of the pond with a wide mesh net leaving the fry behind to grow in domestic settings and potentially be used for spawning the following year, if feed regimens and stocking density in the pond permit such growth.

In natural environments, white catfish mature at greater than 1 year of age. This may be explained by food availability in nature not being as abundant and/or as regularly available as feeding in commercial fish farming environments.

Ovulation of wild brown bullheads was difficult to induce. Aggravating this problem was their extreme vulnerability to columnaris, making it difficult to keep them alive long enough to

spawn once they reached the hatchery. Using a recirculating system may help for spawning wild adults by reducing exposure to disease. If they can be successfully spawned and any fry kept alive, domestication may be critical to develop this species into a model organism. However, for certain environmental applications, reducing their sensitivity to various stressors through domestication could reduce their effectiveness for applications such as a bioindicator.

For the first time, it was demonstrated that white catfish can reach sexual maturity and ovulate at one year of age. This greatly enhances their value as a potential model species for various genetic manipulations for larger, later maturing ictalurid such as channel catfish, flathead catfish, *Pylodictus olivaris*, and blue catfish.



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**Table 1.** Percent ovulation of various types white catfish, *Ictalurus catus* and brown bullhead, *Ameirus nebulosus* induced to ovulate with carp pituitary extract, CPE, and luteinizing hormone releasing hormone analogue, LHRHa, injections and implants in varying spawning environments in 2010 and 2011.

Year	Species	Type	Hormone	IP or IM	# of injections	Experimental unit	Water flow	Individual or communal spawning	n	# fish ovulated	% ovulation
2010	White catfish <i>I. catus</i>	wild	LHRHa EVAC 90µg/kg	IM	1	aquaria	4L/min: constant	Individual	5	5	100
2011	White catfish <i>I. catus</i>	wild	LHRHa CA implant 90µg/kg	IM	1	aquaria	4L/min: constant	Individual	4	0	0
		wild	CPE*	IP	2	aquaria	4L/min: constant	Individual	7	2	28.6
		wild	CPE*	IP	2	aquaria	4L/min: constant	Comm: with male	8	0	0
		wild	CPE/LHRH/CPE/CPE [(2+8mg)+50µg+4mg+4mg/kg]	IP/IM	5	aquaria	4L/min: constant	Comm: with male	4	0	0
		wild	CPE [(2+8mg)+4mg+8mg/kg]	IM/IP	4	aquaria	4L/min: constant	Comm: with male	6	0	0
		wild	CPE*	IP	2	aquaria	4L/min: constant	Com: with female (no divider)	2	0	0
	Brown bullhead <i>A. nebulosus</i>	wild	CPE*	IP	2	aquaria	4L/min: constant	Individual	6	0	0

\*Standard CPE injection was 2mg CPE/kg weight followed by 8mg CPE/kg weight 12 hours later, unless noted to be otherwise.

IM = Intramuscular injection/implant. IP = Intraperitoneal injection.



**Table 2.** Percent ovulation of various types white catfish, *Ictalurus catus* and brown bullhead, *Ameirus nebulosus* induced to ovulate with carp pituitary extract, CPE, and luteinizing hormone releasing hormone analogue, LHRHa, injections and implants in varying spawning environments in 2012.

Year	Species	Type	Hormone	IP or IM	# of injections	Experimental unit	Water flow	Communal or individual	n	# fish ovulated	% ovulation
2012	White <i>I. catus</i>	wild-acclimated	**LHRHa	IP	1	aquaria	4L/min: constant	Individual	6	0	0
	Brown bullhead <i>A. nebulosus</i>	wild	LHRHa	IP	1	aquaria	4L/min: constant	Shared with male	2	0	0
	White <i>I. catus</i>	wild	*CPE	IP	2	tank	6 L/min: constant	communal: all female	3	0	0
			LHRHa	IP	2	tank	6 L/min: constant	communal: all female	4	0	0
		wild-acclimated	CPE	IP	2	tank	6 L/min: constant	communal and separated from males by divider	4	0	0
			LHRHa	IP	2	tank	6 L/min: constant	communal: all female	3	0	0
		domestic	CPE	IP	2	tank	interrupted ††	communal: all female	4	3	75
			LHRHa	IP	2	tank	6 L/min: constant	communal: all female	5	0	0
	Brown bullhead <i>A. nebulosus</i>	wild	LHRHa	IP	2	tank	6 L/min: constant	communal: all female	3	0	0
		wild-acclimated	CPE	IP	2	tank	interrupted ††	communal: all female	2	2	100
			LHRHa	IP	2	tank	6 L/min: constant	communal: all female	2	0	0
	White <i>I. catus</i>	wild-acclimated	CPE	IP	2	tank	interrupted †† †	communal: all female	2	1	50

\*Standard CPE injection was 2mg CPE/kg weight followed by 8mg CPE/kg weight 12 hours later, unless noted to be otherwise.

\*\*Standard LHRHa injection was a single dose of 100µg liquid LHRHa/kg weight, unless noted to be otherwise.

† = Water flow was intentionally interrupted as part of the procedure

†† Marks treatments whose means were statistically different from other treatments ( $\alpha=0.05$ )

During the CPE vs LHRHa experiment, all fish received a preparatory dose of 2mg CPE/kg weight. Treatment injections differed in the resolving dose phase.