GENETIC EFFECTS ON THE PRODUCTION OF CHANNEL CATFISH

(*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus*

furcatus) MALE HYBRID FRY

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GENETIC EFFECTS ON THE PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE HYBRID FRY

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GENETIC EFFECTS ON THE PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE HYBRID FRY

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Joseph Charles Ballenger was born on July 5, 1982 in Seneca, South Carolina. He is the only child of Kenneth and Sherry Ballenger. Joseph graduated from Winthrop University of Rock Hill, South Carolina in 2004 and entered the graduate school of Auburn University in May of 2004. He is married to Erica Nichole Bataller.

THESIS ABSTRACT

GENETIC EFFECTS ON THE PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE HYBRID FRY

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The hybrid from the mating of a female channel catfish (*Ictalurus punctatus*) with a male blue catfish (*Ictalurus furcatus*) exhibits heterosis for various performance traits which would make it attractive to the commercial aquaculture industry. However, the widespread use of the hybrid in the aquaculture industry has been impeded by reproductive isolating mechanisms that prohibit the adequate production of fry. The primary research objective was to evaluate various genotypes of channel catfish females to determine if certain genotypes with blue catfish produced increased numbers of fry when hybridized. Fry output and other reproductive traits of seventeen genotypes of channel catfish females when crossed with blue catfish males were evaluated. The effect of crossbreeding or selection for body weight for channel catfish females was evaluated to determine heterosis, correlated responses or inbreeding depression for hybrid fry production.

No clear effect of strain on percent ovulation, latency period, egg quality, or fecundity was observed, thus any observed differences among strains are likely due to age effects. Strain of female did affect the most important trait, fry/kg female body weight, with genotypes low (162 ± 280 fry/kg) and AU-7 (801 ± 793 fry/kg) being poor performers. Season affected strain differences, and the best channel catfish strain to use during different portions of the spawning season varies.

Three-year-old crossbred females exhibited negative heterosis for ovulation percentage (52.1%) which could be the result of late sexual maturity of the crossbreed. However, earlier experiments indicated that these same fish have early sexual maturity so early sexual maturity for more conventional reproduction does not equate to good reproductive performance. The crossbreed did not exhibit any heterosis for latency period, egg quality, or fecundity. With regards to percent hatch and fry/kg, the crossbreed exhibited performance equal to (4 years of age; 27.4% and 3,393 fry/kg) or less than (3 years of age; 8.4% and 880 fry/kg) the best performing adult. Results indicate no advantage of using this crossbreed to produce hybrid fry compared to the best parental line, AU-1.

Selection for increased body weight had variable effects on reproduction and hybrid fry production. Various lines had increased, decreased, or no change in reproductive output compared to randomly bred controls.

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I. INTRODUCTION

The order Siluriformes is an ancient order of fish that contains the various catfish species found across the world. This order contains 17 families that are found everywhere in the world except in the Arctic and Antarctic (Liu et al. 2003). Siluriformes are found in all three main types of water found across the globe: freshwater, brackish water, and saltwater. They have proven to be a very versatile family that has been cultured by farmers for food across the world. In freshwater habitats of North America, however, only a single family of catfish (Ictaluridae) is found (Tiersch and Goudie 1993). Members of this family are found throughout the United States and are important components to most freshwater habitats.

One particular species of ictalurids, the channel catfish (*Ictalurus punctatus*), has become important to the United States aquaculture industry. The original range of this species was limited to the Mississippi River system in the United States up into southern Canada and southward into northeastern Mexico as well as along the east coast north to Delaware and south to the St. Johns River in Florida (Figure 1). However, due to many introductions outside of their native range, channel catfish are currently found throughout North America in areas that have suitable waters (Dunham and Smitherman 1984).

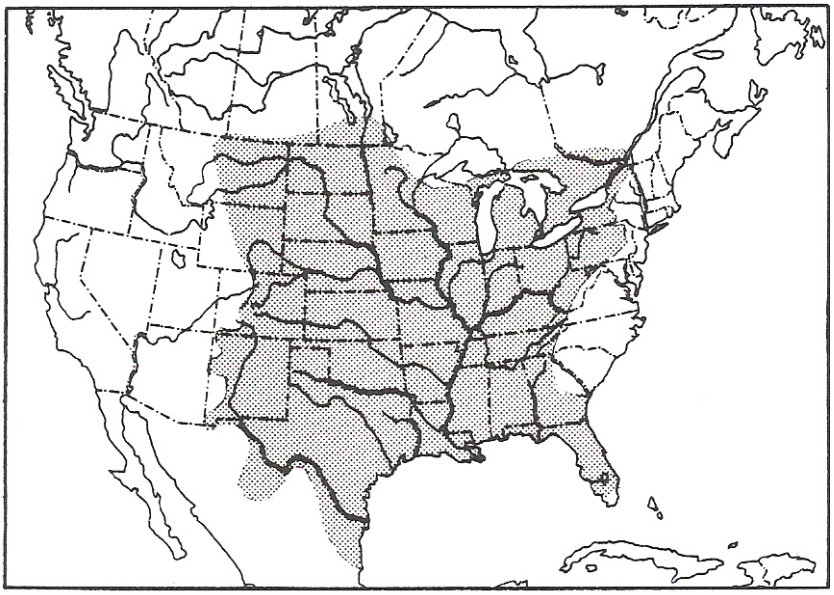

Figure 1: Map of the native distribution of channel catfish (from Dunham and Smitherman 1984).

The first known spawning of channel catfish in captivity occurred in 1892 while as early as 1910 the Kansas State Fish Hatchery at Pratt began propagating channel catfish (Dunham and Smitherman 1984). Soon after, state and federal agencies began growing the channel catfish in various hatcheries across the nation and in the late 1930's, the 1940's and the 1950's the first catfish farms were established in Kansas, Mississippi, and Arkansas (Dunham and Smitherman 1984). However, it was not until the early 1960's that channel catfish culture began to bloom and farms became more widespread (Dunham and Smitherman 1984; Goudie et al. 1993; Tiersch and Goudie 1993; Wolters and Johnson 1994; 1995). Since that time channel catfish culture has come to dominate the aquaculture industry of the United States by surpassing the production of all other food fish (Tiersch et al. 1990; Dunham et al. 1993; Wolters and Johnson 1994; Dunham et al. 1998; Liu et al. 1998; Liu et al. 2003; Rezk et al. 2003).

Today, catfish culture accounts for greater than 50% of all aquaculture production in the United States and is one of the major aquaculture enterprises worldwide (Dunham

et al. 1993; Dunham et al. 1998; Dunham et al. 1999). Over 95% of channel catfish production occurs in the four southeastern states of Mississippi, Arkansas, Alabama, and Louisiana (NASS 2005; Wolters et al. 1996). The catfish industry in the United States continues to expand. In 1990, approximately 163 million kilograms of catfish were processed in the United States and subsequently production has risen to 286 million kilograms, or $480 million in sales by farmers, in 2004 (NASS 2005; Harvey 2005). For 2005, the amount processed by processors is expected to be between 286 and 290 million kilograms (Harvey 2005). This increase in production has been aided by an increase in knowledge regarding the optimum growing conditions needed by channel catfish for maximum efficiency and the application of increasingly intensive culture.

Although channel catfish culture has come to dominate the catfish industry in the United States, the channel catfish is not necessarily the perfect genotype for culture. Channel catfish are the primary culture species in the industry because they grow the fastest to market size of all the ictalurid species studied (Dunham et al. 1993). Other species of ictalurids have characteristics that would be advantageous in a culture system and, therefore, have been considered for possible culture or introgression into channel catfish genomes. These species include the bullhead catfishes (genus *Ameiurus*), which tolerate low dissolved oxygen levels but have extremely slow growth and poor resistance to diseases, the flathead catfish (*Pylodictis olivaris*), which exhibits fast growth, but are cannibalistic and difficult to harvest, the white catfish (*Ameiurus catus*), which exhibit fast early growth, relatively good growth at cold temperatures, and tolerate low dissolved oxygen concentrations, but have slow growth during the food fish production phase, poor dress-out percentage, and poor survival, and the blue catfish (*Ictalurus furcatus*), which

have relatively fast growth, high dress-out percentage, high resistance to enteric septicemia of catfish (ESC), and are easy to harvest via seining, but are considered to have relatively poor disease resistance to other pathogens (Dunham et al. 1993).

Despite investigations of possible aquaculture production of a wide range of ictalurids, the original culture organism, channel catfish, as well as blue catfish exhibit the most promise to the industry and thus research to increase the production of these two species continues (Dunham et al. 1993). Each of these two species exhibit characteristics that would make it superior to the other if not for corresponding agonistic characteristics.

Characteristics of channel catfish that are superior to the blue catfish are growth, tolerance to handling stress, tolerance of high ammonia and high nitrite concentrations in the water, resistance to bacterial infections of *Flexibacter columnaris* and to the parasite *Ichthyophthirius multfiliis*, and earlier sexual maturity (Dunham et al. 1993; Dunham and Argue 2000). However, blue catfish exhibit superior uniformity of growth, are less susceptible to channel catfish virus, proliferative gill disease (Bosworth et al. 2003), and *Edwardsiella ictaluri* (ESC), are easier to seine than channel catfish, and exhibit higher dress-out percentage (Dunham et al. 1993; Dunham and Argue 2000). Neither species can be concluded to be the best for all culture situations (Dunham et al. 1993). Furthermore, the decision as to which species to culture is confounded by the high degree of variability in culture traits that arises due to strain variation within each species (Dunham et al. 1993).

Genetic enhancement is one option for improving the culture traits of catfish. Since channel catfish is the primary culture species, most genetic enhancement research has focused on improving this species. Genetic research to improve the culture traits of

catfish was initiated in the 1960's. Several methods have been employed to try and increase these production characteristics, most notably mass selection of channel catfish for faster growth to market size (Dunham et al. 1987;Dunham and Brummett 1999; Dunham et al. 1999, Rezk et al. 2003), intraspecific breeding programs among different strains of channel catfish (Dunham et al. 1983; Dunham et al. 1987), development of triploid channel catfish (Lilyestrom et al. 1999), and interspecific hybridization (Dunham et al. 1987; Dunham and Brummett 1999; Dunham et al. 1999; Argue et al. 2003). All of these potential techniques, except for the development of triploid channel catfish (Lilyestrom et al. 1999), have resulted in improvements in culture traits of the species.

Selection has been successful in improving the growth of channel catfish (Bondari 1983; Dunham and Smitherman 1983b; Dunham et al. 1987; Dunham and Smitherman 1987; Dunham and Brummett 1999; Dunham et al. 1999) with as much as 50% increase in body weight after four generations of mass selection (Padi 1995). Correlated responses to selection for body weight include improvements in survival, feed conversion ratios, and disease resistance (Dunham and Smitherman 1983b). However, a comparison of two channel catfish lines selected for faster growth for two generations with the channel catfish female X blue catfish male hybrid (CB hybrid) indicated that the hybrid exhibited faster growth than either of the two select lines (Dunham and Brummet 1999).

Success in improving certain characteristics in channel catfish has also been attained by conducting intraspecific breeding. Dunham et al. (1987) reported that the intraspecific crossbreed from the pairing of a Marion strain female channel catfish with a Kansas strain male channel catfish (MK) exhibited faster growth to 100g than the CB hybrid, but by the time the MK reached 500g, the hybrid had achieved the same weight.

In another study, 67% of intraspecific crossbreeds examined exhibited improved growth compared to parental controls, but reciprocal intraspecific hybrids did not grow at the same rates (Dunham and Smitherman 1983a). The MK crossbreed can also be as easy to, or easier to catch by hook- and line than the hybrid catfish (Dunham et al. 1986).

Since the 1960's, a total of twenty-eight different types of ictalurid hybrids have been produced (Dupree and Green 1969; Dupree et al. 1969; Dunham et al. 1987; Goudie et al. 1993; Dunham et al. 2000). These different hybrids were formed from various crosses of channel catfish with other members of the ictalurid family, including the following species: blue catfish, black bullhead (*Ameiurus melas*), yellow bullhead (*A. natalis*), brown bullhead (*Ameiurus nebulosus*), flathead catfish, and white catfish (Goudie et al. 1993). The progeny from these crosses all exhibited characteristics of each of the parents to varying degrees (Goudie et al. 1993). However, of all of the interspecific ictalurid hybrids that had been produced, only the cross between a female channel catfish and a male blue catfish exhibits overdominance for traits desirable for intensive aquaculture (Dunham et al. 1982; Dunham and Smitherman 1983a; Giudice 1966; Dunham et al. 2000).

Interspecific hybridization of female channel catfish and male blue catfish has been successful at improving several culture traits when compared to the two parental species. Improvements include growth uniformity (Giudice 1966; Dunham et al. 1982; Smitherman et al. 1983; Argue et al. 2003), faster growth to market size (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987; Dunham et al. 1990; Dunham and Brummett 1999), tolerance to lower dissolved oxygen concentrations (Dunham et al. 1983), greater resistance to some diseases (Dunham et al.

1990), especially to the major bacterial disease of catfish, ESC, caused by *E. ictaluri* (Wolters et al. 1996), higher dress-out percentage (Smitherman et al. 1983; Argue et al. 2003), higher catchability or seinability (Tave et al. 1981; Dunham et al. 1982; Smitherman et al. 1983; Dunham et al. 1986), greater feed efficiency (Li et al. 2004), and lower mortality rates (Dunham et al. 1987). In fact, the hybrid has yielded increases in body weight of 18-100% over channel catfish (Smitherman et al. 1983; Dunham et al. 1987; Dunham et al. 1990; Dunham and Brummett 1999). These traits are all desirable in an aquaculture organism and in many cases the traits exhibited by the CB hybrid are far superior to most wild or domesticated strains of channel catfish.

Despite this heterosis for culture traits of the hybrid, the widespread use of the hybrid in the commercial catfish industry of the United States has been impeded by the reproductive isolating mechanisms that prevent the parental species from mating and prevents the artificial production of large numbers of embryos that are needed for a commercial farm (Tave and Smitherman 1982; Goudie et al. 1993; Dunham et al. 1998; Dunham et al. 1999; Dunham et al. 2000; Argue et al. 2003; Kristanto 2004). Until improvements are made in the production of the hybrid embryos, the commercial use of the hybrid will be very limited or non-existent because it will not be economically feasible due to the additional costs that are needed to produce sufficient quantities of seed.

Many different production techniques have been used to try and maximize the production of CB hybrid embryos. To maximize the production of hybrid embryos, it is believed that hormone injections are needed to induce final oocyte maturation in females (Bondari 1990), and thus help researchers overcome the reproductive isolating

mechanisms preventing hybridization. When Giudice (1966) first produced the CB hybrid, he spawned the brood stock in pens and aquaria by injecting human chorionic gonadotropin (HCG) intraperitoneally at a rate of 1,543 IU per kilogram of body weight, and he was unsuccessful in stripping eggs from females. In this early attempt, only approximately 30% of the eggs hatched, though he reasoned that the low hatching rate was due to unsuitable conditions in the hatching trough and not because he was trying to produce a hybrid (Giudice 1966).

From Giudice's first attempts to produce CB hybrid embryos, the technique he used was further refined to maximize production of hybrid embryos via pen spawning, aquaria spawning, or artificial fertilization techniques, and eventually a standard recommended dose of 1,100 IU/kg of body weight of HCG was used to induce ovulation (Tave et al. 1981; Tave and Smitherman 1982; Goudie et al. 1992; Goudie et al. 1993; Tiersch and Goudie 1993; Kristanto 2004). In some cases, males were also injected with doses of HCG to try and further aid in artificial production of embryos (Goudie et al. 1993). However, the results from the use of HCG remained inconsistent, with HCG often being unproductive in inducing ovulation and when ovulation did occur, it occurred anytime over a two day period (Kristanto 2004). Therefore, researchers next began looking at the use of another compound, carp pituitary extract (CPE), to induce ovulation in channel catfish for hybrid embryo production (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Dunham and Argue 2000; Dunham et al. 2000; Kristanto 2004).

During a series of experiments that used CPE injections to actuate final oocyte maturation for either pen spawning, aquaria spawning, or artificial fertilization of females, the conclusion was that two CPE injections should be given to each female: a

priming dose of 2mg/kg of female body weight followed by a resolving dose of 8mg/kg approximately 12 hours later (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Dunham and Argue 2000; Dunham et al. 2000; Kristanto 2004). This dosage of CPE improved ovulation rates of females, and made it possible to improve hand stripping techniques and improve hybrid embryo production compared to earlier projects that used HCG to induce spawning (Kristanto 2004). In fact, 90% of the injected females were found to ovulate over a 2-6 hour time period (Kristanto 2004), a marked improvement over the low percentage and extreme variable time period over which ovulation could occur when using HCG. Although improvements were seen, egg quality of the ovulating females remained problematic because many eggs were either under or overripe (Kristanto 2004). Also, consistent potency of CPE may be a problem because of differences in seasons, age, and reproductive readiness at the time that carp are harvested for their pituitaries. Furthermore, both HCG and CPE act directly upon the ovary rather than acting on the organism's pituitary gland to stimulate the release of their own leutenizing hormones that would induce final oocyte maturation and ovulation (Goudie et al. 1992).

Therefore, recent research has examined the efficacy of either purified or synthesized pisces GnRH (gonadotropin releasing hormone) or mammalian LHRH (leutenizing hormone-releasing hormone) (Goudie et al. 1992). GnRH is the major hormone released by the hypothalamus of organisms that controls the release of the pituitary gonadotropins LH (luetinizing hormone) and FSH (follicle stimulating hormone) (Hickman et al. 1998; Starr and Taggert 1998; Dunham 2004). Thus, GnRH is the main hormone that regulates reproduction and gonadal development of many

organisms and thus production of GnRH is at maximum levels in fish while gametes are undergoing maturation (Dunham 2004). Therefore, the artificial injection of natural or synthesized GnRH can induce oocyte maturation and ovulation of fish when trying to spawn them under artificial conditions (Dunham 2004). LH is the actual hormone that is released in the blood stream from the pituitary gland after GnRH stimulation, which induces final oocyte maturation and ovulation in females (Starr and Taggert 1998). In males, high levels of LH in the circulatory system can cause increased sperm production in the testes (Starr and Taggert 1998).

The injection or implantation of LHRH (luteinizing hormone releasing hormone) has been used to induce spawning of a wide range of fish species, including channel catfish, three species of Indian major carps, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), 4 species of European catfish of the genus *Silurus*, a commercial tench (*Tinca tinca*), the common carp (*Cyprinus carpio*), and the swamp eel (*Monopterus albus*) (Kristanto 2004). Furthermore, in a comparison of the effectiveness of HCG and LHRH on the artificial production of CB hybrid embryos, LHRH was at least as effective as HCG at inducing ovulation of channel catfish females (Goudie et al. 1992). In a comparison between the effectiveness of LHRH injections and CPE injections in channel catfish, LHRH induced ovulation 100% of the time while the ovulation rate of females injected with CPE had an ovulation rate of only 60-66.7% (Kristanto 2004). Additionally, Kristanto (2004) concluded that synthetic LHRH was the most promising hormone for production of hybrid embryos via artificial fertilization procedures when he looked at comparisons among embryo production using CPE, synthetic GnRH and synthetic LHRH.

The objectives of the current experiments were to maximize CB hybrid embryo production by determining the effects of strain variation, crossbreeding, and selection for body weight on hybrid embryo production. An optimal genotype of channel catfish may exist that allows for more efficient production of CB hybrid embryos, and would allow greater production of hybrid embryos in the commercial catfish industry.

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II. STRAIN AND LINE VARIATION FOR THE PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE FRY

ABSTRACT

The use of the hybrid from the mating of a channel catfish, *Ictalurus punctatus*, female with a blue catfish, *I. furcatus*, male (CB hybrid) could improve the production efficiency of the catfish aquaculture industry of the United States if problems achieving adequate production of CB hybrid fry are overcome. Genetic enhancement is one option for alleviating this problem and because large amounts of strain variation exist with regards to reproductive traits in channel catfish females, different channel catfish strains could exhibit different abilities to produce CB hybrid fry. CB hybrid fry production and related reproductive traits of different genotypes and ages of channel catfish females were compared. Strain did not have a clear effect on ovulation percentage, latency period, egg quality or fecundity. Strain effects observed for percent ovulation and fecundity were likely caused by differences in ages of females, rather than the strain. Age effects were found for latency period, culling percentage, ovulation rate and spawning percentage. Strain differences were observed ($P=0.03$) for spawning percentage. Strain effects for fry/kg of female body weight were observed, with most strains producing more ($P<0.05$) hybrid fry than genotype low (162 ± 280 fry/kg) or AU-7 (801 ± 793 fry/kg). Time in spawning season was an important factor in determining which channel catfish female strain to use. During the early spawning season, genotype AU-13 (3,390 ± 3,300 fry/kg) was the most productive. Late in the spawning season, genotypes AU-1 control (3,751 ± 3,360) and AU-11 (3,862 ± 2,356) produced the most fry/kg.

Key Words: channel catfish, blue catfish, hybrid catfish, fry production, strain

INTRODUCTION:

The channel catfish (*Ictalurus punctatus*) currently dominates the US catfish industry, accounting for greater than 50% of all aquaculture production (Dunham et al. 1993; Dunham et al. 1998; Dunham et al. 1999). However, the channel catfish is not the best genotype for culture.

The pairing of a channel catfish female with a blue catfish, *I. furcatus*, male (CB hybrid) produces heterosis for several traits (Dupree and Green 1969; Dupree et al. 1969; Dunham et al. 1982; Dunham and Smitherman 1983; Dunham et al. 2000). Improvements over the currently raised channel catfish exist for the following traits: growth uniformity (Giudice 1966; Dunham et al. 1982; Smitherman et al. 1983; Argue et al. 2003), growth to market size (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987; Dunham et al. 1990; Dunham and Brummett 1999), tolerance to low dissolved oxygen concentrations (Dunham et al. 1983b), resistance to some diseases (Dunham et al. 1990), dress-out percentage (Smitherman et al. 1983; Argue et al. 2003), catchability or seineability (Tave et al. 1981; Dunham et al. 1982; Smitherman et al. 1983; Dunham et al. 1986), feed conversion efficiency (Li et al. 2004), and mortality rates (Dunham et al. 1987). The widespread use of the hybrid in the commercial catfish industry has been impeded because of significant problems producing adequate numbers of fry (Tave and Smitherman 1982; Goudie et al. 1993; Dunham et al. 1998; Dunham et al. 1999; Dunham et al. 2000; Argue et al. 2003; Kristanto 2004).

Genetic enhancement is one option for alleviating or overcoming the problem of hybrid fry production. Considerable variation exists for reproductive performance in strains and lines of fish. Large strain differences have been observed for reproductive traits including age of maturity, time of spawning, fecundity and egg size of catfish (Broussard and Stickney 1981, Dunham and Smitherman 1984, Smitherman and Dunham 1985). Females of the Norris and USDA-103 strains typically spawn at two years of age, whereas females of the Kansas and Marion strain are known to require at least a third or fourth year to reach sexual maturity. Other studies have suggested that different strains spawn at different ages, but no significant difference in spawning success (production of viable eggs) was evident (Broussard and Stickney 1981; Dunham et al. 1983a). In a preliminary study, plasma of sexually mature fish from each of the Kansas and USDA-103 strains was obtained in May to determine steroid levels. The spawning success of the USDA-103 fish was higher and levels of plasma testosterone and estradiol were 9-fold higher than those in the Kansas strain (Rex Dunham, personal communication).

The objectives of the current experiment were to determine the effect of strain or line of channel catfish female on hybrid embryo production. If some strains are found to be superior to others at producing CB hybrid fry, it may be possible to make fry production efficient enough to warrant the use of CB hybrids throughout the catfish aquaculture industry. Identifying existing differences in strains/lines, and capitalizing on this variation with strain selection is the first and easiest step in a genetic enhancement program.

MATERIALS AND METHODS:

Experimental Fish and Design

Channel catfish and blue catfish brood stock were maintained at the North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University. Different strains were communally stocked together after being either pit tagged and/or heat branded (Dunham et al. 1982).

A total of seventeen (NWAC-103 (103), Forks albino, low, AU-1, AU-1 control, AU-3, AU-4, AU-5, AU-6, AU-7, AU-7 control, AU-8, AU-9, AU-10, AU-11, AU-12, and AU-13) channel catfish female genotypes were used. Genotypes were communally stocked in twenty 0.1 hectare ponds located at the North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University during the winter of 2003-2004. Females were stocked at an average density of 1,500 kilograms of fish per hectare. The female brood stock was 3-5 years old.

Male blue catfish (*Ictalurus furcatus*) brood stock were communally stocked in ponds. Several different strains of blue catfish were used to fertilize channel catfish female eggs, including the following: Auburn X Rio Grande (AR), Tombigbee (TBB), Craft Auburn, D&B, Rio Grande (RG), Forks D&B, and an unknown blue strain. The male brood stock used varied in age from six to nine years.

Females were harvested by seining approximately two days prior to the expected date of ovulation and were randomly chosen for hormone injection based on secondary sexual characteristics. These characteristics were a well-rounded, distended abdomen,

darkened coloration, and a reddish urogenital area (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Kristanto 2004). Females that did not possess characters indicative of gravidness were not used at the time and were released back into the pond. Upon harvest for a spawning trial, females were transferred to holding tanks until they were injected with luetinizing hormone-releasing hormone analog (LHRHa).

Blue catfish male brood stock were harvested approximately one day prior to ovulation and were chosen based on secondary sexual characteristics. These characteristics were large, muscular pads on the dorsal surface of the head, darkened coloration, well-developed genital papilla, and indications of possible aggressive tendencies toward other males as evidenced by the presence of wounds on and around the head region (Bart et al. 1998; Dunham et al. 1999; Kristanto 2004). These wounds on males during the breeding season are indicative of territoriality and fighting for spawning areas in a pond.

A total of six spawning trials were conducted over a period of four weeks during the 2004 spawning season. Two spawning trials occurred during the week of May 23-29, 2004 (Spawning week 1). A single spawning trial occurred during the week of May 30-June 5, 2004 (Spawning week 2). During the final spawning week (Spawning week 3; June 13-19,2004), three spawning trials were conducted and all remaining fish were removed from each brood pond. Those fish that still did not exhibit well developed secondary sexual characteristics were culled and were considered non- gravid females that would not spawn during the current spawning season.

Preparation and Administration of Hormone Solution

LHRHa (des-Gly^{10},[D-Ala^{6}]LH-RH Ethylamide) was obtained from American Peptide Company in a fine powder form. Its amino acid profile is pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NHEt. Once obtained, a stock solution of LHRHa was made by diluting 1 mg of LHRH in 1 mL of commercial 0.85% physiological saline solution for a stock solution of 1,000 ug/mL. For priming doses, this solution was further diluted to form a stock solution of 200 ug/mL.

Before injection, each fish was weighed and individually bagged in mesh bags. Once the bagged fish were labeled, the bags were clipped to the side of holding tanks using clothes pins. These holding tanks were approximately 3.0m X 0.47m X 0.61m in dimension with a capacity of between 670 and 837 liters of water. Each holding tank was supplied with continuous flow through water and oxygenated with compressed air. Flow rates were maintained at an average rate of 32.8 L/minute. Dissolved oxygen concentration in the tanks averaged 7.4 mg/L. A maximum of 10 bagged fish were placed in each holding tank.

Once bagged, fish were injected intraperitoneally with a priming dose of 20 ug LHRHa/kg of fish body weight. Twelve hours later, a resolving dose of 100 ug LHRHa/kg of fish body weight was injected intraperitoneally (LHRHa powder was 82% active ingredient; priming and resolving doses were actually 16.4 and 82.0 ug/kg, respectively). A standard 21 gauge hypodermic needle was used for all injections. Fish were injected just posterior to the pelvic fins.

Sperm Preparation

Blue catfish males were sacrificed no more than 24 hours prior to use of sperm. The testes of these sacrificed males were removed from the abdominal cavity, taking care to remove the testes in a manner that allowed a minimal amount of mesenteric tissue to remain attached to the extracted testicular tissue. As the testes were removed, they were transferred to a plastic dish, and, if needed, rinsed with saline solution (8.5g of pickling salt/1 L of distilled water) to remove excess blood. The testes were examined and only good quality testicular material was kept to fertilize eggs. Testes of good quality had a whitish coloration and appeared to be full of milt. Sections of testes that were reddish in coloration and that did not appear to be full of milt were subsequently removed and discarded. The remaining testicular material was trimmed, removing any extra mesenteric tissue that remained attached.

Once the testes were trimmed, the weight of the testes was determined. The testes were then placed in a sealed plastic bag so that they could be macerated to release the milt. Maceration was accomplished manually or by mashing with a wooden block. To aid the separation of the milt from the testicular material small amounts of saline solution were added as long as the total amount of saline used did not exceed 10ml of saline per gram of testes.

The resulting saline/sperm solution was strained through a fine mesh strainer and the remaining tissue was discarded. The bag that was used to macerate the testes was rinsed with another small amount of saline solution not letting the total amount of saline used exceed 10mL per gram of testicular tissue. This solution was strained and added to

the first decantation. At this time, enough saline was added so that the final concentration of sperm solution was 10mL of saline per gram of testes macerated.

Artificial Spawning

Beginning approximately 3 hours prior to the time when ovulation was expected, females were periodically checked to see if any eggs had been released, as indicated by eggs attached to the mesh bags. When a female was found ovulating, she was removed from the holding tank still contained in the mesh bag, and placed in a 200ppm tricaine methane sulfate (MS-222) solution. To buffer the pH of the anesthetic, 200ppm of sodium bicarbonate was also added to the tub containing MS-222. The ovulating females were submerged in the solution until the opercula movement slowed. Once opercula movement slowed, the female was removed from the mesh bag and then from the MS-222 solution, and rinsed in freshwater. Females were gently dried and the head wrapped inside a towel and moved to the stripping table.

Eggs were stripped into a dried aluminum pie pan, that had been lightly coated with vegetable shortening. Approximately 200g of eggs were added to each pie pan until the female failed to express eggs. When blood was expressed, the eggs were rinsed with a saline solution (8.5g of salt/1 L of distilled water) to remove the blood. If clumped eggs or eggs connected by ovarian tissue were stripped, these clumps were manually removed. Each pan of eggs was weighed to the nearest gram to determine the approximate number of eggs in the pan.

Fertilization and Incubation

Eggs were fertilized by adding 2.5mL of the sperm solution for every 100g of eggs. The sperm from different males were combined into one stock solution, so eggs

from each female were fertilized by two or more males. Once sperm solution had been added to a pan of eggs, water was added to activate the sperm-egg mixture for fertilization. The eggs were gently swirled, for 1-2 minutes, until thoroughly mixed.

After fertilization, the eggs were allowed to sit undisturbed for at least two minutes to allow the eggs to begin to form an egg mass. Subsequently, the eggs were transferred to a trough filled with pond water for further water hardening. Water hardening was allowed to continue for at least fifteen minutes. After water hardening, the egg masses were transferred to plastic mesh baskets in paddle wheel troughs for incubation. Their position in each trough was recorded and the individual location was labeled.

Once placed in the paddle wheel troughs, the eggs were incubated until hatch. Approximately 24 hours after the eggs were placed in an individual trough, formalin (100 ppm) and copper sulfate (32 ppm) treatments were initiated to control fungal growth. Formalin treatments were always applied first. The eggs were treated a total of three times a day at approximate 8 hour intervals with two treatments of copper sulfate (morning and late evening) and one treatment of formalin (afternoon). Treatments continued until the first fry began to hatch in the trough. If fungus became prevalent on an individual egg mass, fungus was removed, or if the fungus became severe enough, the entire egg mass was removed.

Data Analysis

Spawning traits measured included the percent females culled (number of females culled/total number of females), ovulation rate (number ovulating females/total number of females injected), percent females spawned (number ovulating females/total number of

females in strain), latency period (the time from the priming does of LHRHa until the fish ovulated), and relative fecundity (total number of eggs spawned by a female divided by the female body weight). Fecundity was estimated assuming 65 eggs per gram (Rex Dunham personal communication).

Egg quality characteristics were subjectively described as good (bright, yellow coloration), whiteness, bloodiness, clumpiness, or presence of excess fluid. Subjective evaluations were made for each pan of eggs on a 1 to 5 scale. A score of 5 was assigned for eggs that were deemed of good quality with yellow color that did not have any blood. Eggs that flowed freely but were sticky and had a pale yellow color were assigned a score of 4 while a score of 3 was assigned to free flowing eggs that contained clumps and some blood. A score of 2 was assigned to free flowing eggs that contained clumps, blood and extra fluid while eggs that were white with excessive blood, clumps and extra fluid were assigned a score of 1 (Kristanto 2004).

The hatching percentage of individual egg masses was estimated visually approximately 6-12 hours prior to expected hatch of fry. At this time fry were visibly motile and vigorously spinning in the egg casing making it possible to estimate the percent fry alive in the entire egg mass. Each individual egg mass was weighed. Total number of fry alive was estimated by multiplying the percent alive of an individual egg mass by the total egg mass weight by 40 embryos per gram. Percent hatch was then calculated as the total number of fry produced by an individual female divided by the fecundity of that female. Fry per kilogram of female body weight was calculated as the total number of fry produced by a genotype divided by the body weight of all fish injected belonging to that genotype.

Statistical analyses were performed using Statistical Analysis Software version 9.1. ANOVA was conducted followed by Duncan's Multiple Range Test to compare means among genotypes. Pearson chi-squared goodness of fit test was used to analyze the percentage of females culled, the ovulation rate, and spawning percentage of the various strains.

RESULTS:

Percentage of Females Culled, Ovulation Rate, and Percent Spawning

Of the seventeen genotypes evaluated without respect to age, all had at least 61.7% (61.7-100%) of the fish gravid (fish injected with LHRHa; Table 1). The variation among genotypes with regard to culled fish was different (P=0.0411). Two genotypes had less than 75% of the available fish injected (AU-1 Control and AU-1), although these were only represented by three year-old females (Table 1). Two genotypes (Low and AU-13) had every available fish gravid, and thus injected, while the majority of the remaining genotypes (103, AU-3, AU-4, AU-6, AU-7 Control, AU-8, AU-10, and AU-11) had among 80-90% of the available females gravid (Table 1).

Various genotypes exhibited no difference (P=0.0913) in ovulation rate. Ovulation percentage for individual genotypes ranged from 60.0% (Low) to 100.0% (AU-11 and AU-12; Table 1). However, many of the genotypes exhibited an ovulation percentage between 80 and 90% (103, Forks Albino, AU-4, AU-7, AU-7 Control, and AU-13; Table 1).

When the percentage of spawned females is determined for each of the seventeen genotypes, spawning percentage ranged from 45.7% (AU-1) to 90.0% (AU-13; Table 1). Differences (P=0.0064) among genotypes existed with regards to spawning percentage. Only three genotypes exhibited spawning percentages below 60% (AU-1, AU-1 Control, and AU-10) while four genotypes exhibited a spawning percentage greater than 80% (AU-8, AU-11, AU-12, and AU-13; Table 1). Effects of age existed for culling

Table 1: Percentage of channel catfish, *Ictalurus punctatus*, females gravid, culled, ovulated and spawned by strain during 2004 when induced to spawn with 20 and 100 ug/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa).

Genotype	N	% Gravid	% Culled	% Ovulation	% Spawned
103	48	81.3	18.8	82.1	66.7
Forks Albino	19	94.7	5.3	83.3	79.0
Low	5	100.0	0.0	60.0	60.0
AU-1	35	74.3	25.7	61.5	45.7
AU-1 Control	60	61.7	38.3	78.4	48.3
AU-3	52	86.5	13.5	77.8	67.3
AU-4	36	86.1	13.9	87.1	75.0
AU-5	46	78.3	21.7	77.8	60.9
AU-6	21	81.0	19.1	94.1	76.2
AU-7	24	79.2	20.8	84.2	66.7
AU-7 Control	28	82.1	19.9	87.0	71.4
AU-8	57	89.5	10.5	90.2	80.7
AU-9	29	79.3	20.7	87.0	69.0
AU-10	16	81.3	18.8	61.5	50.0
AU-11	13	84.6	15.4	100.0	84.6
AU-12	10	90.0	10.0	100.0	90.0
AU-13	10	100.0	0.0	80.0	80.0

Strains were different for % culled (P=0.0411) and % spawned (P=0.0064). Strains were not different for % ovulation (P=0.0913; Pearson's Chi-Squared Test). .

percentage (P=0.0005), ovulation percentage (P=0.0060), and for spawning percentage (P<0.0001).

For genotypes represented by three year-old females, the percentage of females considered gravid was not statistically different (P=0.1046). Genotype AU-1 Control had the lowest percentage of females gravid (61.0%) while genotype AU-8 had 100% of the available females injected with LHRHa (Table 2).

Ovulation rate was at least 60.0% (range: 60.0-84.2%) for three year-old females (Table 2). For percent ovulation, genotype AU-1 exhibited the lowest observed percentage of females to ovulate, followed by AU-8 (75.0%) and AU-1 Control (77.8%) (Table 2). These differences among genotypes were not significant (P=0.3165).

Spawning percentage ranged from 44.1% (AU-1) to 75.0% (AU-8) but was not different (P=0.1265; Table 2). Genotypes AU-1 and AU-1 control both had spawning percentages of less than 48% while the other three genotypes had spawning percentages greater than 66% (Table 2).

The percentage of females that were gravid as four year-olds (6 genotypes) is slightly higher (64.3%-100.0%) compared to the five genotypes represented by three year-old females (Table 3). These differences among genotypes were not significant (P=0.2157). Three genotypes exhibited culling percentages between 25 and 37% (103, AU-3, and AU-5) while the three remaining genotypes (AU-4, AU-8, and AU-11) had every available female gravid (Table 3).

Four year-old females exhibited a wider variation (53.3-100.0%) among genotypes for ovulation percentage than three year-old females with AU-3 having only

Table 2: Percentage of 3 year-old channel catfish, *I. punctatus*, females gravid, culled, ovulating and spawned by strain during 2004 when induced to spawn with 20 and 100 ug/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa).

Genotype	**N**	**% Gravid**	**% Culled**	**% Ovulation**	**% Spawned**
103	21	85.7	14.3	83.3	71.4
AU-1	34	73.5	26.5	60.0	44.1
AU-1 Control	59	61.0	39.0	77.8	47.5
AU-7	24	79.2	20.8	84.2	66.7
AU-8	4	100.0	0.0	75.0	75.0

Strains were not different for % ovulation (P=0.3165), % culled (P=0.1046), or % spawning (P=0.1265; Pearson's Chi-Squared Test).

Table 3: Percentage of 4 year-old channel catfish, *I. punctatus*, females gravid, culled, ovulating and spawned by strain during 2004 when induced to spawn with 20 and 100 ug/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa).

Genotype	**N**	**% Gravid**	**% Culled**	**% Ovulation**	**% Spawned**
103	24	75.0	25.0	83.3	62.5
AU-3	20	75.0	25.0	53.3	40.0
AU-4	6	100.0	0.0	83.3	83.3
AU-5	28	64.3	35.7	66.7	42.9
AU-8	8	100.0	0.0	87.5	87.5
AU-11	4	100.0	0.0	100.0	100.0

Strains were not different for % ovulation (P=0.2157) or % culled (P=0.1592). Strains were different for % spawning (P=0.0267; Pearson's Chi-Squared Test).

53.3% of the females injected ovulate (Table 3). Genotype AU-11 had 100.0% of the females injected actually ovulate (Table 3). Despite the wider variation in ovulation percentage, no difference (P=0.2157) in ovulation percentage was evident among strains.

For percent spawning, four year-old females exhibited a range of 40.0% (AU-3) to 100.0% (AU-11; Table 3). In addition to AU-3, AU-5 also had less than 50% of available females release eggs (Table 3). Spawning percentage among strains was different (P=0.0267).

Nine of 13 genotypes represented by five year-old females had at least 85.0% of the females gravid (Table 4). The only four genotypes with lower percentages were AU-4, AU-6, AU-9 and AU-10 (Table 4). Three genotypes (Low, AU-5, and AU-13) had every female gravid and thus injected with LHRHa (Table 4). The differences among strains were not significant (P=0.5018).

Between 50.0% (Low) and 100.0% (AU-11 and AU-12) of the injected females ovulated (Table 4). Other than Low, only one genotype exhibited ovulation percentages less than 80.0% (Table 4). Despite this wide difference, no statistical difference in ovulation percentage existed among genotypes (P=0.1281).

Spawning percentage of five year-old females was not different (P=0.3765) among genotypes. Spawning percentages of strains varied from a low of 50.0% (Low) to a high of 90.0% (AU-12; Table 4). Most of the strains exhibited spawning percentages in excess of 70.0% (Table 4).

Table 4: Percentage of 5 year-old channel catfish, *I. punctatus*, females gravid, culled, ovulating and spawned by strain during 2004 when induced to spawn with 20 and 100 ug/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa).

Genotype	**N**	**% Gravid**	**% Culled**	**% Ovulation**	**% Spawned**
Forks Albino	19	94.7	5.3	83.3	79.0
Low	4	100.0	0.0	50.0	50.0
AU-3	32	93.8	6.3	90.0	84.4
AU-4	30	83.3	16.7	88.0	73.3
AU-5	17	100.0	0.0	88.2	88.2
AU-6	21	81.0	19.1	94.1	76.2
AU-7 Control	27	85.2	14.8	87.0	71.4
AU-8	45	86.7	13.3	92.3	80.0
AU-9	29	79.3	20.7	87.0	69.0
AU-10	16	81.3	18.8	61.5	50.0
AU-11	8	87.5	12.5	100.0	77.8
AU-12	10	90.0	10.0	100.0	90.0
AU-13	10	100.0	0.0	80.0	80.0

Strains were not different for % ovulation (P=0.1281), % culled (P=0.5018), or % spawning (P=0.3765; Pearson's Chi-Squared Test).

Latency Period

Mean latency periods differed ($P<0.05$) among genotypes when data for all ages and spawning weeks were combined, with three distinct groupings apparent (Table 5). Genotype low (mean=45.2 hours) had the longest latency period while genotypes AU-6 (mean=41.3 hours) and AU-8 (mean=41.4 hours) had the shortest latency period (Table 5). Age of the brood stock had an effect ($P=0.0267$) with age four brood stock having the longest latency periods (mean=43.6 hours) and age five females having the shortest (mean=42.4 hours). Age three brood stock latency periods (mean=42.8 hours) were not significantly different from the other two. A significant genotype x age interaction ($P=0.0006$) was also detected.

Also, mean latency period differed ($P=<0.0001$) among different spawning weeks, with mean latency period of all weeks being different ($P<0.05$) from each other. Fish spawned during week two (mean=50.3 hours) had the longest latency periods, followed by those spawned during week 1 (mean=42.1 hours). Fish spawned during week three had the shortest latency periods (mean=40.5 hours). However, no genotype x spawning week interaction ($P=0.9948$) was observed.

For genotypes represented by age three female brood stock, no statistical differences were detected in latency period for any of the strains (Table 5). For age four brood stock, differences ($P<0.05$) among the genotypes were apparent with two groupings (Table 5). Genotypes 103 (mean=45.2 hours) and AU-11 (mean=45.0 hours) had the longest latency periods while strain AU-3 (mean=40.9 hours) had the shortest (Table 5). All other strains were not significantly different from either extreme (Table 5). Differences ($P<0.05$) were evident among genotypes represented by age five brood stock

Table 5: Mean latency period for 3, 4, and 5 year-old channel catfish, *I. punctatus*, females when induced to spawn during the 2004 spawning season using 20 ug/kg and 100 ug/kg injections (priming and resolving doses). Means followed by the same letter are not different (P>0.05) within each column, Duncan's Multiple Range Test.

	Age 3		Age 4		Age 5		All Ages	
Genotype	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**
103	16	43.1 ± 4.1a	15	45.2 ± 5.8a			31	44.0 ± 4.9ab
Forks Albino					17	42.3 ± 3.2bc	17	42.3 ± 3.2bc
Strain Low					2	48.4 ± 3.0a	3	45.2 ± 5.9a
AU-1	16	42.1 ± 2.1a					17	42.5 ± 2.6bc
AU-1 Control	30	42.8 ± 4.4a					31	42.8 ± 4.4abc
AU-3			8	40.9 ± 4.2b	27	42.5 ± 4.9bc	35	42.1 ± 4.7bc
AU-4			5	42.6 ± 5.7ab	22	42.7 ± 3.4bc	27	42.7 ± 3.8bc
AU-5			13	44.6 ± 5.1a	16	41.2 ± 4.5c	30	42.7 ± 4.9bc
AU-6					16	41.3 ± 2.9c	16	41.3 ± 2.9c
AU-7	15	43.7 ± 3.5a					15	43.7 ± 3.5abc
AU-7 Control					20	42.4 ± 4.2bc	20	42.4 ± 4.2bc
AU-8	4	41.8 ± 6.1a	7	42.3 ± 2.7ab	37	41.2 ± 4.2c	48	41.4 ± 4.1c
AU-9					21	44.6 ± 4.7b	21	44.6 ± 4.7ab
AU-10					8	42.2 ± 2.9bc	8	42.2 ± 2.9bc
AU-11			4	45.0 ± 4.9a	7	42.2 ± 4.8bc	11	43.2 ± 4.8abc
AU-12					9	43.0 ± 4.9bc	9	42.2 ± 2.9abc
AU-13					8	44.0 ± 3.9bc	8	44.0 ± 3.9ab

with regards to mean latency period, with three distinct groupings apparent (Table 5). Genotype Low exhibited the longest mean latency period (48.4 hours), and it was statistically different from all twelve other genotypes utilized that were represented by age 5 females (Table 5). Genotype AU-9 (mean=44.6 hours) had the second longest latency period, while genotypes AU-5 (mean=41.2 hours), AU-6 (Mean=41.3 hours) and AU-8 (41.2 hours) had the shortest (Table 5).

During week one (May 23-29, 2004) of spawning, the average water temperature for the week was 28.4^{o}C. At this temperature, the average latency time for all genotypes was 42.1 hours. However, when the fifteen genotypes utilized during week one are compared, no significant effect of genotype (P=0.8797; Table 6)) or age (P=0.8537) is detected. Furthermore, no significant genotype x age interaction (P=0.3029) was observed.

During week two (May 30-June 5, 2004) of spawning the average water temperature was 27.0^{o}C which corresponded to an elevated average latency period (Mean=50.3 hours) of all genotypes compared with week one. However, as with week one, when the 11 genotypes used during week two are analyzed for genotype effects on latency, no differences (P=0.6332) emerge (Table 6). Also, no effect of brood stock age (P=0.8645) or of a genotype x age interaction (P=0.7627) was observed with regards to mean latency period.

During week three (June 13-19, 2004) of the spawning season, the average latency period for all sixteen genotypes spawned was 40.5 hours. This shorter latency period is the result of the average water temperature during this week increasing to 27.8^{o}C as well as the fish accumulating an increasing amount of temperature-days prior

Table 6: Mean latency period for channel catfish, *I. punctatus*, females when induced to spawn during the 2004 spawning season during each spawning week using 20 ug/kg and 100 ug/kg injections (priming and resolving doses). Average water temperatures for the individual weeks were: week 1, 28.4°C, week 2, 27.0°C, and week 3, 27.8°C. There were no significant differences observed among strains in any of the weeks. For individual weeks, any strain that was represented by a single replicate was deleted from analysis.

	Week 1		Week 2		Week 3	
Genotype	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**
103	11	42.3 ± 1.9	7	52.1 ± 1.9	13	41.3 ± 2.9
Forks Albino	6	42.5 ± 2.8			10	41.3 ± 2.3
AU-1	14	42.3 ± 2.2			2	41.1 ± 1.6
AU-1 Control	13	42.0 ± 2.7	5	50.7 ± 2.2	13	40.5 ± 2.5
AU-3	12	42.3 ± 2.9	6	49.8 ± 2.2	17	39.3 ± 3.1
AU-4	15	41.7 ± 2.2	4	49.5 ± 1.7	8	41.2 ± 3.5
AU-5	11	41.8 ± 2.3	7	49.9 ± 2.4	12	39.5 ± 3.2
AU-6	7	40.8 ± 1.9			8	40.9 ± 2.9
AU-7	10	42.4 ± 1.7	2	50.9 ± 3.5	3	43.2 ± 2.0
AU-7 Control	6	42.7 ± 2.2	2	50.4 ± 2.6	12	40.9 ± 3.6
AU-8	17	41.3 ± 2.4	5	49.4 ± 1.5	26	39.9 ± 3.6
AU-9	8	42.4 ± 1.9	7	50.4 ± 1.9	6	40.9 ± 3.0
AU-10					7	41.2 ± 1.1
AU-11	3	43.3 ± 0.7	2	51.2 ± 1.3	6	40.5 ± 3.4
AU-12	3	43.2 ± 0.3			5	41.1 ± 5.0
AU-13	3	42.8 ± 1.6	2	49.0 ± 0.8	3	41.9 ± 4.2

to injection which hypothetically should affect the state of egg maturation and the latency period. As with earlier weeks, no genotype effect (P=0.7548) on mean latency period existed when age of brood stock is ignored (Table 6). Also, no age effect (P=0.6859) or genotype x age interaction (P=0.0707) on mean latency period was detected.

Egg Quality

No differences (P=0.9567) existed in the mean egg quality among genotypes of females that ovulated over all weeks (Table 7). No age (P=0.9790), genotype x age interaction (P=0.9177), or genotype x spawning week interaction (P=0.8035) effects were observed for egg quality. For spawning week one, no significant effect of genotype (P=0.7322; Table 7), age (P=0.3393), or genotype x age interaction (P=0.6069) was observed with regards to egg quality scores. During spawning week two, genotype (P=0.9421; Table 7), age (P=0.3151), or genotype x age interaction (P=0.4900) did not have an effect on mean egg quality scores. For week three, a strain (P=0.8342; Table 7), age, (P=0.8067) or genotype x age interaction (P=0.4177) effect was observed when considering mean egg quality scores.

However, spawning week had a significant effect (P=0.0002) on egg quality. Females spawned during spawning week one (mean=4.0) and spawning week three (mean=3.8) had higher egg quality scores than those spawned during week two (mean=3.4).

Relative Fecundity

When the seventeen genotypes spawned throughout the 2004 spawning season were compared with respect to relative fecundity, strain effects ($P<0.05$) became apparent (Table 8). Three distinct groupings for fecundity emerged among strains with Forks

Table 7: Mean egg quality scores for channel catfish, *I. punctatus*, females when induced to spawn during the 2004 spawning season during each spawning week using 20 ug/kg and 100 ug/kg injections (priming and resolving doses). Average water temperatures for the individual weeks were: week 1, 28.4°C, week 2, 27.0°C, and week 3, 27.8°C. There were no significant differences observed among strains in any of the weeks. For individual weeks, any strain that was represented by a single replicate was deleted from analysis.

	Week 1		Week 2		Week 3		All Weeks	
Genotype	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**
103	10	3.7 ± 0.8	7	3.7 ± 1.0	11	3.9 ± 0.9	28	3.8 ± 0.9
Forks Albino	4	3.5 ± 0.6			10	3.9 ± 0.3	15	3.7 ± 0.5
Strain Low							2	3.5 ± 0.7
AU-1	11	4.2 ± 2.2					13	4.0 ± 0.9
AU-1 Control	11	4.1 ± 0.5	5	3.4 ± 0.5	13	4.1 ± 0.6	29	4.0 ± 0.6
AU-3	12	4.0 ± 0.6	6	3.3 ± 0.5	14	4.0 ± 0.6	32	3.9 ± 0.6
AU-4	14	4.0 ± 1.0	4	3.5 ± 1.7	7	3.7 ± 0.8	25	3.8 ± 1.0
AU-5	8	3.6 ± 1.5	7	3.7 ± 0.8	10	4.0 ± 0.9	25	3.8 ± 1.1
AU-6	7	3.9 ± 0.4			8	3.9 ± 0.6	16	3.8 ± 0.5
AU-7	10	4.0 ± 0.5	2	4.0 ± 1.4	4	3.8 ± 1.3	16	3.9 ± 0.8
AU-7 Control	5	3.8 ± 1.1	2	3.0 ± 0.0	11	3.5 ± 1.2	18	3.6 ± 1.1
AU-8	15	3.9 ± 0.5	5	3.4 ± 1.1	25	3.7 ± 0.9	45	3.7 ± 0.8
AU-9	8	4.5 ± 0.5	7	3.0 ± 1.2	6	3.3 ± 1.2	21	3.7 ± 1.2
AU-10					7	3.6 ± 0.8	8	3.5 ± 0.8
AU-11	3	4.0 ± 0.0	2	3.5 ± 0.7	6	3.5 ± 0.5	11	3.6 ± 0.5
AU-12	3	4.0 ± 0.0			5	3.4 ± 0.9	9	3.6 ± 0.7
AU-13	3	4.3 ± 0.6	2	3.0 ± 1.4	3	3.7 ± 0.6	8	3.8 ± 0.9

Table 8: Mean fecundity (eggs/kg), hatch %, and fry/kg of female channel catfish, *I. punctatus*, brood stock when induced to spawn during the 2004 spawning season using 20 ug/kg and 100 ug/kg injections (priming and resolving doses). Means followed by the same letter are not different (P>0.05) within each column, Duncan's Multiple Range Test.

Genotype	**N**	**Fecundity (eggs/kg) ± SD**	**Hatch % ± SD**	**Fry/Kg ± SD**
103	30	9,186 ± 3,367abc	26.9 ± 28.0ab	2,566 ± 2,580ab
Forks Albino	15	11,367 ± 4,055a	11.9 ± 14.0ab	1,514 ± 2,017abc
Strain Low	3	8,665 ± 2,236abc	1.8 ± 3.2b	162 ± 280c
AU-1	16	6,575 ± 2,863c	33.5 ± 24.8a	2,173 ± 1,739abc
AU-1 Control	29	8,138 ± 3,275bc	36.7 ± 34.9a	3,066 ± 3,141a
AU-3	35	9,207 ± 3,490abc	30.3 ± 44.9ab	2,313 ± 2,473abc
AU-4	27	8,841 ± 3,273abc	22.5 ± 28.2ab	1,869 ± 2,067abc
AU-5	28	9,186 ± 3,248abc	26.8 ± 24.5ab	2,576 ± 2,389ab
AU-6	16	9,479 ± 2,398abc	29.2 ± 24.5ab	2,849 ± 2,670ab
AU-7	16	8,150 ± 2,617bc	10.8 ± 9.8ab	801 ± 793bc
AU-7 Control	20	9,130 ± 3,922abc	10.9 ± 15.1ab	993 ± 1,169abc
AU-8	46	10,202 ± 3,120ab	22.9 ± 35.0ab	2,124 ± 2,400abc
AU-9	20	10,665 ± 3,762ab	35.1 ± 52.2a	2,784 ± 2,327ab
AU-10	8	10,137 ± 4,372ab	12.5 ± 14.5ab (N=7)	1,389 ± 1,629abc (N=7)
AU-11	11	9,790 ± 2,327ab	35.7 ± 32.4a (N=10)	3,079 ± 2,868a (N=10)
AU-12	9	8,646 ± 3,130abc	19.9 ± 27.1ab	1,996 ± 2,721abc
AU-13	8	11,693 ± 3,425a	24.1 ± 23.0ab	3,106 ± 2,890a

Albino (mean=11,367 eggs/kg) and AU-13 (mean=11,693 eggs/kg) exhibiting the highest fecundity and AU-1 (mean=6,575 eggs/kg) the lowest (Table 8). No age (P=0.4774), genotype x age interaction (P=0.2006) or genotype x spawning week interaction (P=0.9712) was observed with regard to fecundity.

Despite no statistically significant effect of age on fecundity, a trend with regards to fecundity appeared, with age 5 females having the highest fecundity rates (mean=9,362 eggs/kg), followed by age 4 females (mean=8,971 eggs/kg) and age 3 females (mean=8,054 eggs/kg).

Spawning week had an effect on the fecundity of females (P=0.0121). Females spawned during spawning week 2 produced more eggs/kg (mean=10,530 eggs/kg) than females spawned during either of the other two spawning weeks. Females spawned during spawning week one had 8,956 eggs/kg and females spawned during week three had 9,164 eggs/kg.

During spawning week one, two distinct groupings of genotypes were observed; Forks Albino (mean=12,005 eggs/kg) and AU-11 (mean=11,835 eggs/kg) having the highest relative fecundities and AU-1 (Mean=7,141 eggs/kg) having the lowest (Table 9). The other twelve genotypes were not significantly different with regards to fecundity from either extreme (Table 9). During spawning week one, no significant effect of age (P=0.7393) or genotype x age interaction (P=0.3119) existed for fecundity.
During spawning week two, three groupings of genotypes emerged with AU-13 (mean=14,424 eggs/kg) having the highest fecundity and AU-7 (mean=6,038 eggs/kg) having the lowest (Table 9). Once again, there was no significant effect of age

Table 9: Mean fecundity of female channel catfish, *I. punctatus*, brood stock when induced to spawn during different spawning weeks of the 2004 spawning season using 20 ug/kg and 100 ug/kg injections (priming and resolving doses). Average water temperatures for the individual weeks were: week 1, 28.4°C, week 2, 27.0°C, and week 3, 27.8°C. Means followed by the same letter are not different (P>0.05) within each column, Duncan's Multiple Range Test.

		Week 1		**Week 2**		**Week 3**
Genotype	**N**	**Fecundity ± SD**	**N**	**Fecundity ± SD**	**N**	**Fecundity ± SD**
103	10	9,951 ± 2,390ab	7	8,719 ± 4,532bc	13	8,732 ± 3,583a
Forks Albino	4	12,005 ± 2,513a			10	10,911 ± 4,756a
AU-1	13	7,141 ± 2,837b			2	3,329 ± 617b
AU-1 Control	11	8,164 ± 3,860ab	5	8,442 ± 3,740bc	13	8,000 ± 2,809a
AU-3	12	9,255 ± 3,977ab	6	10,603 ± 2,584abc	17	8,681 ± 3,448a
AU-4	15	8,459 ± 3,327ab	4	11,257 ± 1,985ab	8	8,350 ± 3,469a
AU-5	9	8,534 ± 2,949ab	7	11,654 ± 2,055ab	12	8,236 ± 3,472a
AU-6	7	10,083 ± 2,015ab			8	8,781 ± 2,767a
AU-7	10	8,239 ± 2,778ab	2	6,038 ± 3,065c	4	8,983 ± 2,006a
AU-7 Control	6	8,637 ± 2,914ab	2	10,293 ± 1,609abc	12	9,183 ± 4,700a
AU-8	16	9,082 ± 3,352ab	5	11,291 ± 1,655ab	25	10,719 ± 3,060a
AU-9	8	10,090 ± 4,593ab	7	11,958 ± 1,551ab	5	9,775 ± 4,702a
AU-10					7	9,656 ± 4,488a
AU-11	3	11,835 ± 1,431a	2	10,459 ± 2,280abc	6	8,545 ± 2,103a
AU-12	3	8,695 ± 2,209ab			5	8,178 ± 3,959a
AU-13	3	10,385 ± 5,251ab	2	14,424 ± 720a	3	11,181 ± 1,670a

(P=0.5635) or genotype x age interaction (P=0.8197) on relative fecundity during this week. Again, despite no significant effect of age, a trend was observed with age five females having the highest fecundities (11,729 eggs/kg), followed by age four females (10,062 eggs/kg), and age three females (8,029 eggs/kg).

During spawning week three, two groupings emerged with AU-1 (Mean=3,329 eggs/kg) having the lowest relative fecundities while all fifteen other strains had higher (P<0.05) fecundities. During spawning week three, age did not have an effect (P=0.2411) on relative fecundity. Also, there was no genotype x age interaction effect (P=0.1946) on fecundity.

Hatch %

Two groupings of genotypes existed with regard to hatch percentage with AU-1 (Mean=33.5%), AU-1 Control (Mean=36.7%), AU-9 (Mean=35.1%), and AU-11 (Mean=35.7%) exhibiting a statistically higher (P<0.05) hatch percentage than Low (Mean=1.8%; Table 8). The other twelve genotypes did not exhibit significantly different hatch percentage than either of the extremes (Table 8).

Again, no age (P=0.2825) or genotype x age interaction (P=0.9410) effect was observed with regards to hatch percentage. In addition, no effect of spawning week (P=0.1372) or genotype x spawning week interaction (P=0.8367) existed for hatch percentage.

Fry/kg

When the seventeen genotypes spawned throughout the 2004 spawning season were compared with respect to fry/kg, three groupings emerge (Table 8). Genotypes AU-1 Control (mean=3,066 fry/kg), AU-11 (mean=3,079 fry/kg), and AU-13 (mean=3,106

fry/kg) produced the most fry/kg (Table 8). Low (mean=162 fry/kg) produced the least fry per kilogram of female body weight injected (Table 8). The remaining thirteen genotypes displayed intermediate values (Table 8).

Age of female brood stock did not have an effect (P=0.1519) on fry/kg production, nor was any genotype x age interaction (P=0.8878) detected. Also, genotype x spawning week interaction did not have a significant effect (P=0.5616) on fry/kg production.

Spawning week had an effect (P=0.0079) on fry/kg production. Females spawned during spawning week two produced more fry/kg (mean=3,053 fry/kg) than females spawned during the other two weeks. Females spawned during week one produced only 1,821 fry/kg on average, while those spawned during spawning week three produced 2,264 fry/kg.

During spawning week one, three distinct groupings of genotypes emerged for fry/kg (Table 10). AU-13 (mean=3,390 fry/kg) produced the most fry/kg while Forks albino (Mean=0.0 fry/kg) produced the least (Table 10). Age of female brood stock (P=0.8257) or genotype x age interaction (P=0.8197) had no effect on the fry/kg produced.

During spawning week two, there was no difference ($P<0.05$) among the eleven genotypes utilized with regards to fry/kg (Table 10). Also, no genotype x age interaction effect (P=0.7385) on fry/kg of female. However, age did affect (P=0.0263) fry/kg. Age five females produced the most fry/kg (mean=3,957 fry/kg), followed by age four females (mean=2,390 fry/kg), and age three females (mean=2,262 fry/kg).

Table 10: Mean fry production of female channel catfish, *I. punctatus*, brood stock when induced to spawn during different spawning weeks of the 2004 spawning season using 20 ug/kg and 100 ug/kg injections (priming and resolving doses). Average water temperatures for the individual weeks were: week 1 was 28.4°C, week 2 was 27.0°C, and week 3 was 27.8°C. Means followed by the same letter are not different (P>0.05) within each column, Duncan's Multiple Range Test.

	Week 1		Week 2		Week 3	
Genotype	N	Fry/kg ± SD	N	Fry/kg ± SD	N	Fry/kg ± SD
103	10	1,791 ± 1,813abc	7	3,136 ± 3,887a	13	2,973 ± 2,379ab
Forks Albino	4	0 ± 0c			10	2,019 ± 2,217ab
AU-1	13	2,339 ± 1,818abc			2	811 ± 1,147ab
AU-1 Control	11	2,842 ± 3,462abc	5	1,780 ± 1,227a	13	3,751 ± 3,360a
AU-3	12	1,629 ± 2,089abc	6	5,081 ± 1,381a	17	1,820 ± 2,432ab
AU-4	15	1,805 ± 2,117abc	4	1,953 ± 2,284a	8	1,949 ± 2,158ab
AU-5	9	2,079 ± 2,082abc	7	3,620 ± 2,957a	12	2,338 ± 2,265ab
AU-6	7	3,204 ± 3,559ab			8	2,676 ± 2,020ab
AU-7	10	824 ± 769abc	2	1,604 ± 895a	4	343 ± 628bb
AU-7 Control	6	813 ± 771abc	2	2,386 ± 670a	12	851 ± 1,288ab
AU-8	16	1,744 ± 2,388abc	5	2,138 ± 2,211a	25	2,371 ± 2,500ab
AU-9	8	2,266 ± 2,688abc	7	3,103 ± 2,370a	5	3,167 ± 1,934ab
AU-10					6	1,356 ± 1,785ab
AU-11	3	218 ± 378bc	2	5,413 ± 3,609a	5	3,862 ± 2,356a
AU-12	3	433 ± 750abc			5	2,833 ± 3,429ab
AU-13	3	3,390 ± 3,300a	2	5,425 ± 3,613a	3	1,275 ± 1,138ab

During spawning week three, two groupings of genotypes were apparent for fry/kg (Table 10). AU-1 Control and AU-11 produced ($P<0.05$) more fry/kg than AU-7 (Table 10). The other thirteen genotypes were not significantly different from either extreme (Table 10). However, as with spawning week one, there was no effect of age ($P=0.3959$) or genotype x age interaction ($P=0.4873$) on fry production.

DISCUSSION

A significant difference existed among genotypes with regard to culling percentage and spawning percentage when all ages of fish were considered together, although there was not a difference with regards to ovulation percentage. However, two (AU-1 and AU-1 Control) of the three strains that exhibited spawning percentages equal to or less than 50% were only represented by three year-old females. These fish have a history of late sexual maturity. Some of the differences may be partially due to age effects. However, examination of strains represented by three year-olds, reveals a difference among strains with regard to ovulation percentage, so there is a genetic effect on ovulation.

When strains represented by only three year-old females are compared, only two strains exhibited fairly low ovulation and spawning percentages, those being AU-1 and AU-1 control. The remaining three strains all exhibited ovulation percentages that compared favorable to the ovulation percentages of Kristanto (2004) when utilizing various injections of LHRHa to induce spawning. Strain differences were apparent for age of sexual maturation.

When strains represented by only four and five year old females are analyzed, there was no significant difference with regard to culling percentage, ovulation percentage, or spawning percentage for either age. However, if the level of significance is raised to P=0.055, genetic differences exist for spawning percentages for 4-year-old

females. Large observed differences were found for 5-year-old females, 50.0-100% ovulation and 50.0-90.0 % spawned, but variation was too great to demonstrate statistically significant differences.

The ovulation percentages obtained in the current study were similar to those obtained by Kristanto (2004). Also, the ovulation percentages compared favorably to the ovulation percentages obtained through the use of other ovulating agents, such as carp pituitary extract, CPE, (Dunham et al. 1998; Dunham and Argue 2000; Dunham et al. 2000; Kristanto 2004), human chorionic gonadotropin, HCG, (Goudie et al. 1992), and Ovaprim (Goudie et al. 1992; Kristanto 2004).

Genotypic differences were observed for latency period for all ages, all ages combined, and also among spawning weeks for all strains combined. However, strains were not represented in equal numbers during each spawning week and water temperature has a strong effect on latency period of channel catfish injected with LHRHa (Kristanto 2004). The data indicates that latency period is strongly affected by a combination of the temperature of the time of spawning as well as the accumulated temperature degree days prior to induced spawning. Water temperature has also been an important correlate to latency period in other species like sutchi catfish, *Pangasius hypophthalamus*, and common carp, *Cyprinus carpio* (Kristanto 2004).

When the mean latency periods of strains spawned during each of the three spawning weeks were compared to each other, there was no effect of strain or age of female brood stock. This result was found for each of the spawning weeks. Thus, differences seen during the base analysis was not an effect of genotype or age.

When females of all ages spawned during the 2004 spawning season were pooled, there was no evidence of a significant effect of strain or female age on egg quality. The eggs stripped from females of all strains were of consistent high quality as indicated by high mean scores and fairly low standard deviations. The use of LHRHa appears to alleviate the problem of variable egg quality, as was also suggested by Kristanto (2004). This is a major improvement, as one of the major problems of using CPE as the ovulating agent had been the highly variable quality of eggs obtained (Dunham et al. 2000; Kristanto 2004).

For all three of the spawning weeks, there was no indication of any strain, age, or strain x age interactive effects on egg quality. However, there did appear to be a general trend of eggs released at higher average water temperatures being of higher quality. Eggs released during spawning weeks one (28.4^{o}C) and three (27.8^{o}C) tended to be of higher quality than those during spawning week two (27.0^{o}C) even though these differences in water temperature were subtle.

For relative fecundity, when females of all ages and spawning weeks were analyzed, there was a strain effect. This was only a subtle difference considering that only three strains (AU-1, AU-1 Control, AU-7) produced significantly fewer eggs than strain AU-13, which had the highest fecundity rate. Furthermore, these differences could be explained by the age of female brood stock instead of actual strain effects. Although statistical analysis did not indicate a strain effect, it was curious that all of the strains with significantly lower fecundity rates were all represented by only age three females. It could be that with these three strains of females, at three years of age fecundities are severely reduced and that by four years of age no difference between the best producing

strain and the worst producing strain would be seen. This idea is strengthened by the observation that there were significant differences among ages with regard to fecundity. Regardless of the reason for the observed difference, all strains analyzed exhibited fecundity rates per kilogram of female body weight that compare to those observed by other researchers (Dunham and Argue 2000; Kristanto 2004). In the case of AU-1 control, AU-3, and AU-4, our observed fecundities exceeded those obtained by other researchers (Dunham et al. 1983a; Dunham and Smitherman 1984).

Spawning week affected relative fecundity. There was no significant effect of age during any of the individual spawning weeks although a general trend of older females having higher fecundities was observed. However, during each of the weeks, a strain effect existed. But once again, these effects are probably more likely the result of a subtle age effect and not due to actual differences in strains. This is because during each of the weeks, the strains that had significantly lower fecundities (Week 1: AU-1; Week 2: 103, AU-1 Control, AU-7; Week 3: AU-1) were predominately only represented by three year old individuals. Strains AU-1, AU-1 Control, and AU-7 were only represented by three year olds, while approximately 50% of 103 females were age three, while the rest were age four. The observed fecundities of all strains met or exceeded those observed by other researchers (Dunham and Argue 2000; Kristanto 2004).

The expectation was that age differences among strains would affect fecundity because fecundity usually decreases with size. Since age differences are not apparent, the differences could have been masked because of environmental factors. In particular, a possible explanation for the lack of an age effect is because of the competition of younger fish with older, big fish in the communally stocked ponds. Because the older, bigger fish

were possibly more competitive for food resources, this could have caused the fecundity of younger females to be lower than expected.

Strain, age, spawning week, strain x age interaction, and strain x spawning week interaction had an effect on hatch percentage. Only one strain, Low, exhibited significantly lower hatching rates. Excluding strain Low, all other strains exhibited CB fry hatch percentages in excess of 10.9% (range: 10.9-36.7%) which is greater than the CB fry hatch observed by Dunham et al. (2000) when they produced hybrids using CPE as the ovulating agent. Although, hatching percentage was often up to 3-fold different between certain genotypes, virtually no statistically significant differences were obtained because of high variability. The hatching percentages were similar to the hatch percentages observed for channel catfish female—channel catfish male pairings (Dunham et al. 2000). Also, hatch percentages were similar to those obtained by Giudice (1966) when using HCG, and Kristanto (2004) who used Ovaprim, CPE, and various concentrations of LHRHa to produce hybrids.

Other researchers have also found no difference in the hatch percentages of AU-1 control, AU-3, or AU-4 when crossed with channel catfish males (Dunham et al. 1983a; Smitherman and Dunham 1984). However, the observed hatch of AU-3 and AU-6 was less than that observed by Broussard and Stickney (1981) when they evaluated the hatch percentage of channel catfish females paired with channel catfish males in spawning containers without injection of ovulating agents.

For fry/kg, line AU-1 control and line AU-13 produced more fry/kg than strains low and AU-7. Additionally, six strains (103, AU-1 Control, AU-5, AU-6, AU-9, and AU-13) exhibited fry production rates in excess to those obtained by strain Low. Strains

AU-1 control and AU-13 appear to cross better with blue catfish males to produce CB hybrid fry. However, for all strains other than strain Low, CB hybrid fry production exceeded that achieved (Dunham et al. 2000) when CPE was used to induce spawning of channel catfish females, and all but three strains (low, AU-7, and AU-7 Control) exceed the production of channel catfish fry when CPE was used as the ovulating agent (Dunham et al. 2000). In addition, the fry production values of all but the above mentioned three strains were comparable to those obtained by Kristanto (2004) when he used LHRHa as the ovulating agent.

Significant effects of strains were seen in both week one and week three. During spawning week one, AU-13 produced more fry than Forks albino and AU-11 and AU-6 produced more CB hybrid fry than Forks albino. AU-13 and AU-6 may be lines that should be used during the early spawning season. Late in the spawning season it may be appropriate to use other strains, considering that during spawning week three strains AU-1 control and AU-11 had the greatest CB hybrid fry production, being significantly higher than that produced using strain AU-1.

There was not a clear effect of strain on ovulation percentage, latency period, egg quality, or fecundity. The strain effects observed for ovulation percentages and fecundity rates are more likely caused by differences in ages of females, rather than the strain itself although no age effects were indicated overall. Poorly performing strains for both traits were represented primarily by three year-old females. For at least a few strains, females were not fully mature at three years of age, and will exhibit lower ovulation percentages and fecundity rates.

Strain affected the most important trait, fry/kg female body weight with most strains producing more hybrid fry than strain low or AU-7. Certain strains may be more useful in producing CB hybrid fry than others during different portions of the spawning season. Although difference were not seen with regard to CB fry production during spawning week two, during the early spawning season it appears that it would be useful to utilize strain AU-13 for hybrid production and not to use Forks albino. Furthermore, late in the spawning season, it would be beneficial not to use AU-1 as the channel catfish female used for CB hybrid fry production, but possibly use AU-1 control and AU-11. Utilizing certain strains or lines of channel catfish females is a genetic management tool that can enhance the production of channel-blue hybrid catfish fry.

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III. THE EFFECT OF USING CROSSBRED CHANNEL CATFISH (*Ictalurus punctatus*) FEMALES TO PRODUCE CHANNEL CATFISH FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE HYBRID FRY

ABSTRACT

The main problem preventing the widespread use of the channel catfish, *Ictalurus puncatatus*, female X blue catfish, *Ictalurus furcatus*, male hybrid catfish (CB hybrid) has been inadequate production of commercial numbers of fry in an efficient manner. One method of overcoming this barrier is the use of genetic enhancement programs, and in particular crossbreeding. In the current experiment, a crossbreed, AU-7 X AU-1 was compared to its parental lines to determine if any reproductive benefits were achieved through the use of the crossbreed when forming CB hybrid catfish. The crossbreed (52.1%) actually exhibited lower ovulation percentages than the parental lines (AU-7=83.3%; AU-1=63.2%), suggesting negative heterosis by the crossbreed for age at maturity. For latency period, egg quality, and fecundity, there was no maternal heterosis exhibited by the crossbreed, with parental lines performing as well or better than the crossbreed. With regards to hatch percentage and fry/kg, the crossbreed exhibited performance equal to (four years of age; 27.4 % and 3,393 fry/kg) or less than (three years of age; 8.4% and 880 fry/kg) the best performing adult. There was no advantage to using this crossbred line to form CB hybrid, since the best overall two year performance was exhibited by parental line AU-1.

Key Words: channel catfish, crossbreeding, reproductive traits, blue catfish, hybrid

INTRODUCTION:

The culture of channel catfish in the United States accounts for greater than 50% of all aquaculture production in the US (Dunham et al. 1993; Dunham et al. 1998; Dunham et al. 1999) and accounted for over $480 million dollars in sales by farmers in 2004 (Harvey 2005; NASS 2005). Despite this status, profitability of the catfish industry has been marginal the past five years because of production insufficiencies, marketing and competition from imports. Production of channel catfish, *Ictalurus punctatus*, female X blue catfish, *Ictalurus furcatus*, male hybrids (CB hybrid) would alleviate this problem.

Interspecific ictalurid hybrids could exhibit heterosis for various culture traits. Although a total of twenty-eight different types of ictalurid hybrids have been produced since the 1960's (Dunham et al. 1987; Goudie et al. 1993; Dunham et al. 2000), the only interspecific ictalurid hybrid that has shown heterosis for culture traits is the channel catfish female hybridized with a blue catfish male (Dupree et al. 1969; Dunham and Smitherman 1981; Dunham et al. 1982; 2000). This interspecific hybrid has shown improvements in growth uniformity (Giudice 1966; Dunham et al. 1982; Smitherman et al. 1983; Argue et al. 2003), growth to market size (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987; Dunham et al. 1990; Dunham and Brummett 1999), tolerance to low dissolved oxygen concentrations (Dunham et al. 1983a), disease resistance (Dunham et al. 1990), dress-out percentages (Smitherman et al. 1983; Argue et al. 2003), catchability or seineability (Tave et al. 1981;

Dunham et al. 1982; Smitherman et al. 1983; Dunham et al. 1986), feed efficiency (Li et al. 2004), and mortality rates (Dunham et al. 1987).

Despite these advantageous qualities, the widespread use of the interspecific CB hybrid in the commercial catfish industry has been impeded by the reproductive isolating mechanisms that separate the two species. Because of these reproductive isolating mechanisms it has been difficult to produce the large numbers of fry that are needed for commercial production of the CB hybrid (Tave and Smitherman 1982; Goudie et al. 1993; Dunham et al. 1998; Dunham et al. 1999; Dunham et al. 2000; Argue et al. 2003; Kristanto 2004).

Initially, to produce CB hybrid fry, gravid channel catfish females were induced to ovulate using human chorionic gonadotropin (HCG) (Giudice 1966; Tave et al. 1981; Tave and Smitherman 1982; Goudie et al. 1992; Goudie et al. 1993; Tiersch and Goudie 1993; Kristanto 2004). Though induced ovulation did occur using HCG, the results remained inconsistent with low ovulation percentages commonly occurring and, when ovulation did occur, it occurred over a period of two days (Kristanto 2004). Later researchers began looking at using carp pituitary extract (CPE) as the compound to induce ovulation in gravid channel catfish females (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Dunham and Argue 2000; Dunham et al. 2000; Kristanto 2004). The use of CPE improved ovulation rates of females, and narrowed the ovulation period, making it possible to improve hand stripping techniques and fry production (Kristanto 2004). Despite these improvements, significant problems remained, the most important being low egg quality of the ovulating females (Kristanto 2004). Recently, Kristanto (2004) compared CPE, synthetic GnRH and synthetic LHRHa, and concluded that

synthetic LHRH was the most promising hormone for the production of hybrid embryos via artificial fertilization.

Although great improvements have been made producing CB hybrid fry using artificial fertilization techniques, fry production is still limiting the use of CB hybrids in the commercial catfish industry. Genetic approaches may help solve this problem.

Crossbreeding can result in heterotic reproductive performance, and crossbred females are commonly used in the livestock industry to benefit from maternal heterosis for reproductive traits (Lasley 1978). In the case of channel catfish, crossbred brood stock can exhibit heterotic reproductive performance, and age X genotype interactions are possible. Crossbreeding can improve spawning rates of channel catfish in ponds (Dunham et al. 1983b), and early sexual maturity was expressed by crossbred channel catfish (Smitherman and Dunham 1985). Gall (1969, 1975) observed heterosis for egg size in crossbred rainbow trout.

Another application of crossbreeding is to counteract the effects of inbreeding. The detrimental effects of inbreeding depression when the inbreeding coefficient, F, reaches approximately 0.25 is well documented in cultured aquatic organisms resulting in depression of growth, production, survival and reproduction of up to 30% or more (Dunham et al. 2001). One generation of inbreeding (F = 0.25) in channel catfish decreased egg hatchability by 15.2% and survival to 90 days by 5.3% in Mississippi, and increased number of days needed for eggs to hatch in Georgia (Smitherman and Dunham 1985). Spawning rates, egg size, survival, and hatch of inbred fish were unaffected in Alabama for a more diverse strain, however, fecundity of inbred brood stock (F = 0.25) was actually higher than for randomly bred controls. This apparent anomaly was caused

by the fact that several weak inbred lines either did not reproduce or died leaving some high performing lines to reproduce and inflate the means. As was the case in catfish (Bondari and Dunham 1987), inbreeding adversely affected reproduction in rainbow trout (Gjerde et al. 1983, Kincaid 1983), and family response or the extent of inbreeding depression for each family was variable.

Our objective was to compare crossbred and parental line channel catfish females for their ability to produce hybrid embryos. Utilization of crossbred females may be a mechanism to improve hybrid embryo production.

MATERIALS AND METHODS:

Experimental Fish and Design

Channel catfish and blue catfish brood stock were maintained at the North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University. Different strains were communally stocked together after being either pit tagged and/or heat branded (Dunham et al. 1982).

In the current study, a crossbred channel catfish female (AU-7 X AU-1) along with the parental lines (AU-1 and AU-7) were compared during the 2004 and 2005 spawning season. Both of the parental lines had been selected for increased body weight for six generations. The genotypes were communally stocked in 0.1 hectare ponds located at the North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University during the winter of 2003-2004. In these brood stock ponds, females were stocked at an average density of 1,500 kg of fish/ha. All females used for the study were three years of age.

Male blue catfish (*Ictalurus punctatus*) brood stock used in 2004 were communally stocked in ponds. Several different strains of blue catfish were used to fertilize channel catfish female eggs, including the following: Auburn X Rio Grande (AR), Tombigbee (TBB), Craft Auburn, D&B, Rio Grande (RG), Forks D&B, and an unknown blue strain. The male brood stock used varied in age from six to nine years.

In 2005, the channel catfish 4 year-old-females utilized the previous year were stocked in brood stock ponds at the Fish Genetics Research Unit (0.25 hectare ponds).

Females were stocked at a rate of 1,750 kg/hectare in each pond. Blue catfish males for the 2005 spawning season originated from either the North Auburn Fisheries Research Unit or from Eagle Aquaculture farm. Blue males used belong to one of the following strains: Rio Grande (RG), D&B, Forks D&B, and Auburn X Rio Grande. The male brood stock used varied in age from six to nine years.

Females were harvested by seining approximately two days prior to the expected date of ovulation and were randomly chosen for hormone injection based on secondary sexual characteristics. These characteristics were a well-rounded, distended abdomen, darkened coloration, and a reddish urogenital area (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Kristanto 2004). Females that did not possess characters indicative of gravidness were not used at the time and were released back into the pond. Upon harvest for a spawning trial, females were transferred to holding tanks until they were injected with luetinizing hormone-releasing hormone analog (LHRHa). The same procedure was used in 2005 to select female brood stock for the spawning trial.

For both spawning seasons, blue catfish male brood stock were harvested approximately one day prior to ovulation and were chosen based on secondary sexual characteristics. These characteristics were large, muscular pads on the dorsal surface of the head, darkened coloration, well-developed genital papilla, and indications of possible aggressive tendencies toward other males as evidenced by the presence of wounds in and around the head region (Bart et al. 1998; Dunham et al. 1999; Kristanto 2004). These wounds on males during the breeding season are indicative of territoriality and fighting for spawning areas among neighboring males in a pond.

A total of six spawning trials were conducted over a period of 4 weeks during the 2004 spawning season. Two spawning trials occurred during the week of May 23-29, 2004 (spawning week 1). A single spawning trial occurred during the week of May 30-June 5, 2004 (spawning week 2). During the final spawning week (spawning week 3; June 13-19, 2004), three spawning trials were conducted and all remaining fish were removed from each brood pond. Those fish that still did not exhibit well developed secondary sexual characteristics were culled and were considered not gravid that would not spawn during the current spawning season.

For the 2005 spawning season, one spawning trial was conducted. All females were removed from the ponds and implanted with LHRHa.

Preparation and Administration of Hormone Solution

LHRHa, des-Gly^{10},[D-Ala^{6}]LH-RH Ethylamide, was obtained from American Peptide Company in a fine powder form. Its amino acid profile is pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NHEt. Once obtained, a stock solution of LHRHa was made by diluting 1mg of LHRH in 1ml of commercial 0.85% physiological saline solution for a stock solution of 1000 ug/ml. For priming doses, this solution was further diluted to form a stock solution of 200ug/ml.

Before injection, each fish was weighed and individually bagged in mesh bags. Each bag was labeled with the fish's weight and individual fish number. This fish number was used to track individual fish and subsequent egg masses. Once the bagged fish were labeled, the bags were clipped to the side of holding tanks using clothes pins. These holding tanks were approximately 3.0m X 0.47m X 0.61m in dimension with a capacity of between 670 and 837 liters of water. Each holding tank was supplied with

continuous flow through water and oxygenated with compressed air. Flow rates were maintained at an average rate of 32.8 L/minute, and dissolved oxygen concentration in the tanks averaged 7.4 mg/L. A maximum of 10 bagged fish were placed in each holding tank. The main purpose for limiting ten fish per holding tank was to allow adequate space between bags so that entanglement among bags did not occur.

In 2004, bagged fish were intraperitoneally injected with a priming dose of 16.4 ug LHRHa/kg of fish body weight. Twelve hours later, a resolving dose of 82.0 ug LHRHa/kg of fish body weight was intraperitoneally injected (LHRHa powder was 82% active ingredient). A standard 21 gauge hypodermic needle was used for all injections. Fish were injected just posterior to the pelvic fins.

In 2005, females were placed in 20 gallon aquaria once the ovulating agent had been administered. The ovulating agent was administered by inserting a 100 ug LHRHa implant (Mylonas and Zohar 2001). The implant was placed approximately 3 cm posterior of the dorsal fin and 4 cm ventral to the top of the fish.

Sperm Preparation

Blue catfish males were sacrificed no more than 24 hours prior to use. The testes of these sacrificed males were removed from the abdominal cavity, taking care to remove the testes in a manner that allowed a minimal amount of mesenteric tissue to remain attached to the extracted testicular tissue. As the testes were removed, they were transferred to a plastic dish, and if needed, rinsed with saline solution (8.5g of pickling salt/1 L of distilled water) to remove excess blood. The testes were examined and only good quality testicular material was kept to fertilize eggs. Testes of good quality had a whitish coloration and appeared to be full of milt. Sections of testes that were reddish in

coloration and that did not appear to be full of milt were subsequently removed and discarded. The remaining testicular material was trimmed, removing any extra mesenteric tissue that remained attached.

Once trimmed, the weight of the testes were determined. The testes were then placed in a sealed plastic bag so that they could be macerated to release the milt. Maceration was accomplished manually or by mashing with a wooden block. To aid the separation of the milt from the testicular material small amounts of saline solution were added as long as the total amount of saline used did not exceed 10ml of saline per gram of testes.

In 2004, the resulting saline/sperm solution was strained through a fine mesh strainer and the remaining tissue was discarded. The bag that was used to macerate the testes was rinsed with another small amount of saline solution not letting the total amount of saline used exceed 10mL per gram of testicular tissue. This solution was strained and added to the first decantation. At this time, enough saline was added so that the final concentration of sperm solution was 10mL of saline per gram of testes macerated.

In 2005, the sperm preparation procedure slightly deviated from that used in 2004. Once enough saline solution was added to bring the concentration to 10ml of saline per gram of testes macerated, the solution was then analyzed using a spectrophotometer to determine the concentration of sperm. Once the concentration of the sperm was determined, it could then be further diluted so that approximately $6.5 \text{ X } 10^7$ sperm per 100 eggs was used.

Artificial Spawning

Beginning approximately 3 hours prior to the time when ovulation was expected, females were periodically checked to see if any eggs had been released as indicated by eggs attached to the mesh bags. When a female was found ovulating, she was removed from the holding tank still contained in the mesh bag, and placed in a 200 ppm tricaine methane sulfate (MS-222) solution. To buffer the pH of the anesthetic, 200 ppm of sodium bicarbonate was also added to the tub containing MS-222. The ovulating females were submerged in the solution until the opercula movement slowed. Once opercula movement slowed, the female was removed from the mesh bag and then from the MS-222 solution, and rinsed in freshwater. Females were gently dried and the head wrapped inside a towel and moved to the stripping table. During this entire period, whenever a fish was held out of the water care was taken to plug the vent with a finger to prevent the premature release of eggs.

Eggs were stripped into a dried aluminum pie pan, which had been lightly coated with vegetable shortening. Approximately 200g of eggs were added to each pie pan until the female failed to express eggs. When blood was expressed, the eggs were rinsed with a saline solution (8.5g of salt/1 L of distilled water) to remove the blood. If clumped eggs or eggs connected by ovarian tissue were stripped, these clumps were manually removed. Each pan of eggs was weighed to the nearest gram to determine the approximate number of eggs in the pan.

Fertilization and Incubation

In 2004, fertilization of eggs was accomplished by adding 2.5mL of the sperm solution for every 100g of eggs. The sperm from different males were combined into one

stock solution, so eggs from each female were fertilized by two or more males. Once sperm solution had been added to a pan of eggs, water was added to activate the sperm-egg mixture for fertilization. The eggs were gently swirled, for 1-2 minutes, until thoroughly mixed.

After fertilization, the eggs were allowed to sit undisturbed for at least two minutes to allow the eggs to begin to form an egg mass. Subsequently, the eggs were transferred to a trough filled with pond water for further water hardening. Water hardening was allowed to continue for at least fifteen minutes. After water hardening, the egg masses were transferred to plastic mesh baskets in paddle wheel troughs for incubation. Their position in each trough was recorded and the individual location was labeled. This was done to ensure that each individual egg mass could be traced back to the correct female for data analysis.

Once placed in the paddle wheel troughs, the eggs were incubated until hatch. Approximately 24 hours after the eggs were placed in an individual trough, formalin and copper sulfate treatments were initiated to control fungal growth. Formalin treatments (100 ppm) were always applied first. The eggs were treated a total of three times a day at approximate 8 hour intervals with two treatments of copper sulfate (morning and late evening, 32 ppm) and one treatment of formalin (afternoon). Treatments continued until the first fry began to hatch in the trough. If fungus became prevalent on an individual egg mass, fungus was removed, or if the fungus became severe enough, the entire egg mass was removed.

In 2005, the treatment of eggs deviated slightly from that used in 2004. Based on data from Small and Chatakondi (2006) that illustrated that hatch rate could be greatly

improved if the eggs were not treated with formalin or copper sulfate during a particular critical period of time after fertilization, the eggs during 2005 were not treated between 42 and 46 hours after fertilization when the water was 28^{o}C. By converting this time to temperature hours, the critical period is 1,176-1,288 temperature -hours. However, once this critical period elapsed, eggs were once again treated approximately every eight hours, using copper sulfate in the early morning and late evening and formalin in the afternoon. Eggs were treated until the first fry began to hatch in the trough.

Data Analysis

Spawning traits measured included the percent females culled (number of females culled/total number of females), ovulation rate (number ovulating females/total number of females injected), percent females spawned (number ovulating females/total number of females in strain), latency period (the time from the priming does of LHRHa until the fish ovulated), and relative fecundity (total number of eggs spawned by a female divided by the female body weight).

Egg quality characteristics were subjectively described as good (bright, yellow coloration), whiteness, bloodiness, clumpiness, or presence of excess fluid. Subjective evaluations were made for each pan of eggs on a 1 to 5 scale. A score of 5 was assigned for eggs that were deemed of good quality with yellow color that did not have any blood. Eggs that flowed freely but were sticky and had a pale yellow color were assigned a score of 4 while a score of 3 was assigned to free flowing eggs that contained clumps and some blood. A score of 2 was assigned to free flowing eggs that contained clumps, blood and extra fluid while eggs that were white with excessive blood, clumps and extra fluid were assigned a score of 1 (Kristanto 2004).

The hatching percentage of individual egg masses was estimated visually approximately 6-12 hours prior to expected hatch of fry. At this time fry were visibly motile and vigorously spinning in the egg casing making it possible to estimate the percentage of fry alive in the entire egg mass. Each individual egg mass was weighed. Total number of fry alive was estimated by multiplying the percent alive of an individual egg mass by the total egg mass weight by 40 eggs per gram. Hatch percentage was then calculated as the total number of fry produced by an individual female divided by the fecundity of that female. Fry per kilogram of female body weight was calculated as the total number of fry produced by a genotype divided by the body weight of all fish injected belonging to that genotype.

Statistical analyses were performed using either Statistical Analysis Software version 9.1 or Microsoft Excel 2003. ANOVA was conducted followed by Duncan's Multiple Range Test to compare means among genotypes. Pearson chi-squared goodness of fit test was used to analyze the percentage of females culled, the ovulation rate, and spawning percentage of the various strains.

RESULTS

Three-Year-Old Female Brood Fish

Culling, Ovulation, and Spawning percentage

During the 2004 spawning season when the experimental fish were three-years-old, the crossbreed AU-7 x AU-1, was not significantly different from its parental lines for culling percentage (P=0.6038). All genotypes had between 68 and 79% of the fish gravid (Table 11).

However, there were differences among the genotypes for percent ovulation (P=0.0308). The crossbreed AU-7 x AU-1 had less than 50% of injected fish ovulate while the two parental lines had greater than 60% ovulation rate (Table 11). Parental line AU-7 had greater than 80% of fish injected with LHRHa ovulate (Table 11).

Differences also existed among genotypes for spawning percentage (P=0.0203). The crossbreed AU-7 x AU-1 had only 31.9% of its females produce eggs while both parental lines had greater than 44% of available females give eggs (Table 11). AU-7 had the highest percentage of females spawn (66.7%; Table 11).

Latency Period

When the latency period of the crossbreed AU-7 x AU-1 and its two parental lines were compared for three year-old fish spawned during all spawning weeks of 2004, genotype did not have an effect on mean latency period (P=0.1285). All lines had mean latency periods between 42 and 44 hours after the priming dose of LHRHa (Table 12). No genotype x spawning week interaction existed for latency period (P=0.3462).

Table 11: Percentage of three year-old females gravid, culled, ovulated, and spawned for AU-1, AU-7, and the crossbreed AU-7 female x AU-1 male channel catfish, *Ictalurus punctatus*, during the 2004 spawning season when induced to spawn with 20 and 100 ug/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa).

Genotype	N	% Gravid	% Culled	% Ovulation	% Spawned
AU-1	34	73.5	26.5	60.0	44.1
AU-7	24	79.2	20.8	84.2	66.7
AU-7 x AU-1	47	68.1	31.9	46.9	31.9

Ovulation % (P=0.0308) and spawning % (P=0.0203) was significantly different among genotypes. Strains were not different for % culled (P=0.6038) (Pearson's Chi-Squared Test).

Spawning week affected mean latency period ($P<0.0001$).

During spawning week one (May 23-29, 2004), the average water temperature for the week was 27.0°C. This corresponded to a mean latency period for all genotypes combined of 42.7 hours. However, there was no effect of genotype on mean latency period ($P=0.2787$; Table 12).

During spawning week two (May 30-June 5, 2004), the average water temperature for the week was 28.4°C. This corresponded to a mean latency period for all genotypes combined of 50.9 hours, which was significantly longer than the mean latency period of all genotypes combined during spawning week one. However, because only line AU-7 spawned during week two, no analysis comparing strains could be conducted (Table 12).

During spawning week three (June 13-19, 2004), the average water temperature for the week was 27.8°C. This corresponded to a mean latency period for all lines combined of 42.0 hours, which was significantly shorter than the mean latency period of all genotypes combined during spawning week two. However, it was not significantly different from the combined mean latency period of all genotypes during spawning week one. Also, when comparing the lines spawned during week three, there was no effect of strain on mean latency period ($P=0.5710$; Table 12).

Egg Quality

When the egg quality scores of the crossbreed and its parental lines spawned during all weeks of 2004 are compared, there was no strain effect on egg quality scores ($P=0.5509$; Table 13). Spawning week did not have an effect on egg quality scores

Table 12: Mean latency period for AU-1, AU-7 and the crossbreed AU-7 female x AU-1 male channel catfish, *I. punctatus*, females when induced to spawn during the 2004 spawning season. No differences among genotypes were found during any of the periods analyzed. Mean water temperatures during individual spawning weeks were: week 1 was 27.0°C, week 2 was 28.4°C, and week 3 was 27.8°C. There were no significant differences among strains for any spawning week, nor when all weeks were combined.

	Week 1		Week 2		Week 3		All Weeks	
Genotype	**N**	**Mean +/- SD**	**N**	**Mean +/- SD**	**N**	**Mean +/- SD**	**N**	**Mean +/- SD**
AU-1	14	42.3 ± 2.2			2	41.1 ± 1.6	16	42.1 ± 2.1
AU-7	10	42.4 ± 1.7	2	50.9 ± 3.5	3	43.2 ± 2.0	15	43.7 ± 3.5
AU-7 x AU-1	10	43.6 ± 2.1			7	41.8 ± 2.5	17	42.8 ± 2.4

Table 13: Ovulation %, mean latency period, egg quality score, fecundity (eggs/kg), hatch %, and fry/kg for AU-1, AU-7 and the crossbreed AU-7 female x AU-1 male channel catfish, *I. punctatus*, females when induced to spawn during the 2004, 2005, and 2004 and 2005 spawning season combined. Means followed by the same letter are not different (P>0.05) within each column, Duncan's Multiple Range Test.

	Ovulation %		Latency Period		Egg Quality		Fecundity (Eggs/kg)		Hatch %		Fry/kg	
Genotype	N	%	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Three-Year Old-Females												
AU-1	25	60.0	16	42.1 ± 2.1a	12	4.0 ± 1.0a	15	6,633 ± 2,954a	15	32.5 ± 25.3a	15	2,135 ± 1,794a
AU-7	19	84.2	15	43.7 ± 3.5a	16	3.9 ± 0.8a	16	8,150 ± 4,829a	16	10.8 ± 9.8b	16	801 ± 793b
AU-7 x AU-1	22	46.9	17	42.8 ± 2.4a	13	3.7 ± 0.6a	15	7,317 ± 4,829a	15	8.4 ± 15.5b	15	880 ± 1,591b
Four-Year-Old Females												
AU-1	13	69.2	9	73.4 ± 9.5a	2	5.0 ± 0.0a	9	10,581 ± 2,366a	9	32.5 ± 31.4a	9	3,772 ± 3,443a
AU-7	5	80.0	3	55.1 ± 3.2b			4	12,579 ± 3,196a	4	9.9 ± 15.9a	4	1.592 ± 2.773a
AU-7 x AU-1	16	62.5	10	65.7 ± 7.7a	5	4.6 ± 0.9a	10	12,230 ± 3,038a	10	27.4 ± 26.3a	10	3,393 ± 3,002a
Pooled Years												
AU-1	38	63.2	25	53.4 ± 16.4a	14	4.1 ± 0.9a	24	8,113 ± 3,327a	24	32.5 ± 27.1a	24	2,749 ± 2,595a
AU-7	24	83.3	18	45.6 ± 5.5b	16	3.9 ± 0.8a	20	9,036 ± 3,213a	20	10.7 ± 10.8b	20	959 ± 1,348b
AU-7 x AU-1	48	52.1	27	51.3 ± 12.3a	18	3.9 ± 0.8a	25	9,282 ± 4,806a	25	16.0 ± 21.0b	25	1,885 ± 2,537ab

Ovulation % was different among genotypes for three-year-old (P=0.0308) and when data from three-year-old and four-year-old females are pooled (P=0.0353). Ovulation % was not different for four-year-old females (P=0.7568; Pearson's Chi-Squared Test)

(P=0.3608). No genotype x spawning week interaction existed for egg quality scores (P=0.0633).

Relative Fecundity

As with egg quality, when the fecundity (eggs/kg) of all females spawned during 2004 are compared, there was no effect due to genotype (P=0.4745; Table 13). No effect of spawning week (P=0.1213) or genotype x spawning week interaction effect was observed with regard to fecundity (P=0.2033).

Hatch percentage

Differences in hatch percentage among genotypes were observed (P=0.0007). The parental line AU-1 had (P<0.05) higher hatch percentages (32.5%) than either the crossbred or the other parental line AU-7 (Table 13). No spawning week (P=0.3047) or genotype x spawning week interactions (P=0.9277) were observed.

Fry/kg

Genotype had a significant effect on the number of fry produced per kilogram of female body weight (P=0.0258) when all spawning weeks are combined. The parental line AU-1 (Mean=2,135 fry/kg) had higher fry/kg production than either the crossbreed AU-7 x AU-1 or the other parental line AU-7 (Table 13). The two latter genotypes were not significantly different from each other (Table 13). Spawning week (P=0.2572) and strain x spawning week interactions (P=0.7222) did not have an effect on fry per kilogram of female body weight.

Four-Year-Old Female Brood Fish

Ovulation percentage

Genotype did not have an effect (P=0.7568) on ovulation percentage. All genotypes exhibited ovulation percentages in excess of 60% with AU-1 (62.5%) having the lowest percentage of females ovulate and AU-7 (80.0%) had the highest percentage of females ovulate (Table 13).

Latency Period

Mean latency period was different among the different genotypes of females (P=0.0092). AU-1 (73.4 hours) and AU-7 X AU-1 (65.7 hours) had longer mean latency periods than line AU-7 (55.1 hours; Table 13).

Egg Quality

No effect due to genotype (P=0.5761) existed for egg quality. Only two genotypes were compared for this trait because no egg quality observations were made for parental line AU-7. The crossbreed AU-7 x AU-1 and the parental line AU-1 each had mean egg quality scores in excess of 4.5 (Table 13).

Relative Fecundity

Relative fecundity was not different among genotypes (P=0.3590). All genotypes exhibited mean relative fecundities in excess of 10,000 eggs per kilogram of female body weight (Table 13).

Hatch percentage

For hatch percentage, genotype of female did not have an effect (P=0.3984). Parental line AU-7 (9.9%) had the lowest observed hatch percentage while the other parental line, AU-1 (Mean=32.5%), exhibited the highest observed mean (Table 13).

Fry/kg

Finally, with regards to fry/kg production, when the crossbreed AU-7 X AU-1 is compared to its parental strains, genotype does not have any effect (P=0.5154). Mean fry/kg ranged from a low of 1,592, for parental line AU-7, to a high of 3,772, for parental line AU-1 (Table 13).

Pooled Three-Year and Four-Year Old Brood Fish

Ovulation percentage

Genotype had an effect (P=0.0353) on ovulation percentage when the data from three-year-old and four-year-old females were combined. The crossbreed (52.1%) had the lowest observed ovulation percentage while parental line AU-7 (83.3%) had the highest ovulation percentage (Table 13).

Latency Period

Genotype (P<0.0001), age (P<0.0001), and a genotype X age interaction (P<0.0001) all had a significant effect on latency period. The crossbreed and the parental line AU-1 had a longer latency period than AU-7 (Table 13). Also, age four females (67.4 hours) had a longer latency period than age three females (42.9 hours).

Egg Quality

For the pooled data, genotype (P=0.7260) and genotype X age interaction (P=0.9005) had no effect on egg quality (Table 13). However, age had an effect (P=0.0090), with age four females (mean=4.7) having a mean egg quality score approximately one point higher than age three females (mean=3.9).

Relative Fecundity

Age of female brood fish had an effect on relative fecundity when the data from three-year-old and four-year-old females are pooled ($P<0.0001$). Age four females (mean=11,645 eggs/kg) produced approximately 4,000 more eggs/kg than three-year-old females (7,384 eggs/kg). However, there was neither a genotype ($P=0.4499$) nor a genotype X age interaction ($P=0.8871$) effect on fecundity (Table 13).

Hatch percentage

When the data was pooled from the 2004 and 2005 spawning seasons, there was no effect of age ($P=0.1713$) or genotype X age interaction ($P=0.2218$) on hatch percentage. An effect of genotype on hatch percentage ($P=0.0022$) was observed. The parental line AU-1 exhibited between two and three times the hatch of line AU-7 and the crossbreed AU-7 x AU-1 (Table 13).

Fry/kg

Also an effect of genotype ($P=0.0273$) on fry/kg existed. Parental line AU-1 (2,749 fry/kg) produced significantly greater amount of fry/kg than the other parental line (959 fry/kg), while the crossbreed was intermediate (1,885 fry/kg; Table 13). Age also had an on fry/kg ($P=0.0018$). Age four females produced 3,228 fry/kg, while age three individuals only produced 1,262 fry/kg.

DISCUSSION:

During the 2004 spawning season when the fish were three years old, there was no statistical difference in culling percentage among the crossbreed AU-7 x AU-1, and the parental lines, but significant differences were evident in both ovulation percentage and spawning percentage. The crossbred genotype had a lower ovulation percentage and spawning percentage, negative heterosis, than either of the two parental genotypes. While the ovulation percentages of the parental genotypes compared favorably to the results obtained by Kristanto (2004), the ovulation percentages of the crossbreed was lower. The results of the parental genotypes were similar to the ovulation percentages of channel catfish females obtained through the use of carp pituitary extract as the ovulating agent (Dunham et al. 1998; Dunham and Argue 2000; Dunham et al. 2000). The parental strains also exhibited ovulation rates similar to those obtained by Goudie et al. (1992) when they used both Ovaprim (another form of LHRH with a dopamine inhibitor) and human chorionic gonadotropin (HCG). Thus, it appears that for at least the parental genotypes, that LHRHa was at least effective as other ovulating agents at inducing ovulation.

The lower ovulation and spawning percentages obtained from the crossbreed could be the result of negative heterosis for reproductive performance, or delayed sexual maturation. This is a surprising result as Dunham et al. (1983b) observed the opposite effect for intraspecific spawning in pens within ponds when ancestors of these lines were

compared to their crossbreed. In that study, the crossbreed exhibited positive heterosis for early sexual maturation, but there was no difference among genotypes at four years of age. The negative heterosis for early sexual maturity in the current study is the more likely explanation since the same females spawned as four- year-olds during the 2005 spawning season, and had ovulation percentage intermediate to the parental lines, AU-7 and AU-1. Six generations of selection for body weight in the parental lines has apparently altered their genotypes such that combining ability for reproductive performance has been altered. Alternatively, this may be a genotype X environment interaction for reproductive performance as the original study was intraspecific spawning in pens, whereas the current study was induced spawning and artificial fertilization to produce interspecific hybrids.

Regardless of spawning period, no effect of genotype was observed on latency period when induced to spawn with a 20 ug/kg priming dose of lueitenizing hormone-releasing hormone analog (LHRHa) followed by a resolving dose of 100 ug/kg of LHRHa at three years of age. It appears that the water temperature is the major determinant for latency period. As water temperature increases, naturally the latency period from the time of priming dose of LHRHa becomes shorter. It has also been found that in Sutchi catfish, *Pangasius hypophthalamus,* females that are induced to spawn with Ovaprim, there is a positive correlation between water temperature and latency period (Kristanto 2004). When common carp, *Cyprinus carpio*, are induced to spawn with a 20 ug/kg injection of LHRHa there is a significant effect of water temperature on latency period (Kristanto 2004). Another factor that may cause shorter latency periods during the

latter portion of the spawning season is the accumulation of temperature degree days which causes maturation of ova and thus would shorten latency periods.

However, in 2005 when the fish were older and a 100 ug implant of the ovulating agent LHRHa was used to induce spawning, there was a significant effect of genotype on latency period. The crossbreed had an intermediate latency period (65.7 hours) that was between those of the parental lines (AU-1=73.4 hours and AU-7=55.1 hours) and, there was a much wider variation in latency periods for each of the genotypes. This increased variation in latency period occurs when using implants of LHRHa during the first half of the spawning season (Rex Dunham personal communication). Apparently, there is either a genotype X age, genotype X delivery method or genotype X temperature interaction for latency period in channel catfish females. Also, in general, higher dosages of LHRHa inducing shorter latency periods (Kristanto 2004).

The observed differences in latency period when the data from three-year-old and four-year-old females were combined, was likely an artifact due to the fact that all four-year-old individuals were spawned in 2005, which has already been shown to have much longer latency periods than the three-year-old individuals spawned in 2004. Thus, the effect on latency period is caused not by the genotype of individual female, but more likely is caused either by an age, temperature, or delivery method of LHRHa effect.

Egg quality scores of the crossbreed AU-7 x AU-1 and its parental lines were high both years. The eggs in 2005 could have been of higher quality because females used were older. Alternatively, egg quality could have been improved due to the use of an implant of LHRHa instead of priming and resolving doses of LHRHa. Additionally, the

three-year-old females had to compete with older females during preparation whereas the four-year-old females were prepared by themselves.

Genotype of female did not have an effect on fecundity during either of the two spawning seasons nor when the data from the spawning seasons are combined. Fecundity of the genotypes during the 2004 spawning season varied between 6,600-8,200 eggs/kg of female body weight. In 2005, the fecundity of females belonging to all genotypes was higher (range: 10,600-12,600 eggs/kg) than that seen during the 2004 spawning season. Either increased sexual maturity or some environmental difference was the cause of this increase as relative fecundity usually decreases with size. Other researchers, with strains of similar breeding history, have also found that there has been no improvement in fecundity when using a crossbreed (Dunham et al. 1983b; Dunham and Smitherman 1984). However, the same researchers found that when using the crossbreeds AR and ARMK, there was an improvement in fecundity over the parental lines (Dunham et al. 1983b; Dunham and Smitherman 1984). Crossbreeding has variable effects on fecundity as it does for virtually all traits.

Hatch percentage and fry/kg were interrelated. During the 2004 spawning season, there was a significant effect of genotype on the hatching percentages of eggs with parental strain AU-1 having significantly higher hatches than either the parental line AU-7 or the crossbreed. Genotype AU-1 had a hatching rate similar to that seen with aquaria spawned channel catfish that have been injected with CPE (Dunham et al. 2000). Hatch percentage was not significantly different during 2005, but ranged from a low of 9.9% (AU-7) to a high of 32.5% (AU-1). The observed result was that AU-1 had three times

the hatch of AU-7 both years, triple that of the crossbreed when three-years-old, but very similar to the crossbreed at four-years of age.

When the data from three-year-old and four-year-old females was combined, there was also an effect of genotype on hatch. The parental line AU-1 had a hatch percentage between two and three times that seen by either the parental line AU-7 or the crossbreed AU-7 x AU-1. Dunham et al. (1983b) also found that when they used crossbreeds to produce channel catfish fry there was no improvement in hatch percentage for the crossbreed over the parental lines.

Results were essentially parallel for fry/kg. Three-year-old females belonging to the genotypes AU-7 (801 fry/kg) and AU-7 x AU-1 (880 fry/kg) produced significantly fewer fry/kg of female body weight than genotype AU-1 (2,135 fry/kg). In fact, the fry production of genotype AU-1 was greater than that observed among channel catfish spawned in aquaria after being injected with CPE (Dunham et al. 2000). The fry production of the crossbreed AU-7 x AU-1 and the parental line AU-7 is virtually identical to the fry production of channel catfish spawned by Dunham et al. (2000). All fry production values examined in the current experiment were higher than those seen for CB hybrid production by Dunham et al. (2000).

No statistical significant differences in fry production were observed during the 2005 spawning season once all females had achieved four years of age. During this spawning season, fry per kilogram of female body weight varied from a minimum of 1,600 (AU-7) to a maximum of 3,800 (AU-1). Similar to hatch percentage, the observed result was that AU-1 had 2-3 times the fry production of AU-7 both years, triple that of the crossbreed when three-years-old, but very similar to the crossbreed at four-years of

age. Also, similar to hatch percentage, when the data is pooled for three-year-old and four-year-old females, AU-1 produces approximatly three times as much fry/kg than AU-7, and about two times as many as AU-7 x AU-1.

One explanation for the difference in hatch percentages and fry production among the genotypes is a genetic maternal effect with regards to these reproductive traits. The crossbreed and the maternal genotype, AU-7, produced virtually identical hatching percentages and fry production values during the 2004 spawning season. However, the maternal effect may only be evident at younger ages, since once females achieved four years of age, all genotypes examined have statistically identical hatching percentages and fry production.

Reproductive traits often exhibit heterosis and can be improved with crossbreeding (Gall 1969; Gall 1975; Lasley 1978, Dunham et al. 1983b; Smitherman and Dunham 1985). There was no heterosis observed in this experiment. At three years of age, there was the possibility of dominance from the poorer performing AU-7 parent, but this genetic mechanism was not evident at four years of age.

The parental lines were potentially inbred and may have been exhibiting inbreeding depression for reproductive performance. The data from the three-year olds does not support this hypothesis as means for the crossbreed are the same as the poorest parent, and crossbreeding should correct inbreeding depression. If AU-7 was experiencing inbreeding depression, data from the 4-year-old brood fish would support correction of the inbreeding depression for AU-7, but not for AU-1. Contradictory, to what was observed, the effects of heterosis and inbreeding are usually more dramatic at younger ages.

If we consider the results from both years and the data from combined years, there is no benefit for hybrid fry production by using crossbred channel catfish females for these strains as dams. The best two year performance would be obtained from using the AU-1 line as dams.

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IV. THE EFFECT OF MASS SELECTION FOR BODY WEIGHT ON PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALES X BLUE CATFISH (*Ictalurus furcatus*) MALE HYBRID FRY

ABSTRACT

Six lines of channel catfish, *Ictalurus punctatus,* females that had been selected for increased body weight for 6-7 generations were compared to randomly bred controls for channel catfish female X blue catfish, *I. furcatus,* hybrid embryo production. No indication of either a negative correlated response or inbreeding depression for ovulation percentage, latency period, egg quality, fecundity, hatching percentage, or fry/kg was evident. In some cases, a possible positive correlated response between direct selection for body weight and ovulation percentage, fecundity, hatching percentage, and fry/kg occurred. With regards to the interrelated traits hatch percentage and fry/kg, the selected line AU-6 (29.2% and 2,849 fry/kg) exhibited over a 2.5X increase in comparison to the randomly bred control AU-7 (10.9% and 993 fry/kg). Also, similar results were seen when comparing the selected line AU-3 (30.4% and 2,313 fry/kg) to AU-7 control (10.9% and 993 fry/kg).

Key Words: Selection; Channel Catfish, Hybrid Catfish, Inbreeding depression, correlated response.

INTRODUCTION:

The interspecific hybrid from the mating of a channel catfish, *Ictalurus punctatus,* female with a blue catfish, *I. furcatus,* male (CB hybrid) exhibits heterosis for several culture traits (Dupree et al. 1969; Dunham et al. 1982; Dunham and Smitherman 1981; Dunham et al. 2000). The CB hybrid has improved growth uniformity (Giudice 1966; Dunham et al. 1982; Smitherman et al. 1983; Argue et al. 2003), growth to market size (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987; Dunham et al. 1990; Dunham and Brummett 1999), tolerance to low dissolved oxygen concentrations (Dunham et al. 1983), disease resistance (Dunham et al. 1990), dress-out percentage (Smitherman et al. 1983; Argue et al. 2003), catchability or seineability (Tave et al. 1981; Dunham et al. 1982; Smitherman et al. 1983; Dunham et al. 1986), feed efficiency (Li et al. 2004), and mortality rates (Dunham et al. 1987).

Mass selection has greatly improved growth characteristics of channel catfish (Bondari 1983; Dunham and Smitherman 1983;1984; Dunham et al.. 1987; Dunham and Brummett 1999; Dunham et al. 1999; Rezk et al. 2003). Correlated responses to a single generation of selection for body weight in channel catfish include improved survival, feed conversion ratios, disease resistance, and fecundity (Dunham and Smitherman 1983).

Selection for increased body weight could result in positive or negative correlated responses to selection for reproductive traits including hybrid embryo output.

Additionally, mass selection is a mild inbreeding program which after several generations of selection could result in enough cumulated inbreeding to cause inbreeding depression for reproductive traits.

Our goal was to determine if any correlated response for body weight on hybrid embryo production existed. This information is needed for design of appropriate genetic enhancement programs and to maximize hybrid fry production.

MATERIALS AND METHODS:

Experimental Fish and Design

Channel catfish brood stock were maintained at the North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University. Different lines were communally stocked together after being either pit tagged and/or heat branded so that the environmental differences affecting each strain could be minimized (Dunham et al. 1982).

A total of six channel catfish female lines (AU-1, AU-3, AU-4, AU-6, AU-7, and AU-8) that had been selected for increased body weight for 6-7 generations were individually compared to one of three randomly bred controls (AU-1 control, AU-7 control or AU-1 control + AU-7 control) during the 2004 and 2005 spawning seasons. The control that the selected line was compared was based on which control had the most similar genetic history. For the 2004 spawning season, the different genotypes were communally stocked in twenty different 0.1 hectare ponds located at the North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University during the winter of 2003-2004. In these brood stock ponds, females were stocked at an average density of 1,500 kg of fish per hectare. Females ranged in age from three- to-five years.

Male blue catfish brood stock used in 2004 were communally stocked in ponds. Several different strains of blue catfish were used to fertilize channel catfish females,

including the following: Auburn X Rio Grande (AR), Tombigbee (TBB), Craft Auburn, D&B, Rio Grande (RG), Forks D&B, and an unknown blue strain. The male brood stock used varied in age from six to nine years.

In 2005, the channel catfish 4 -year-old females utilized belonged to only one set, AU-1 and AU-1 control (same individuals from previous year). They were stocked in five brood stock ponds at the Fish Genetics Research Unit (0.25 hectare ponds). Females were stocked at a rate of 1,750 kg/hectare in each pond. Blue catfish males for the 2005 spawning season originated from either the North Auburn Fisheries Research Unit or from Eagle Aquaculture farm. Blue males used belong to one of the following strains: Rio Grande (RG), D&B, Forks D&B, and Auburn X Rio Grande. The male brood stock used varied in age from six to nine years.

Females were harvested by seining approximately two days prior to the expected date of ovulation and were randomly chosen for hormone injection based on secondary sexual characteristics. These characteristics were a well-rounded, distended abdomen, darkened coloration, and a reddish urogenital area (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Kristanto 2004). Females that did not possess characters indicative of gravidness were not used at the time and were released back into the pond. Upon harvest for a spawning trial, females were transferred to holding tanks until they were injected with luetinizing hormone-releasing hormone analog (LHRHa). The same procedure was used in 2005 to select female brood stock for the different spawning trials.

For both spawning seasons, blue catfish male brood stock were harvested approximately one day prior to ovulation and were chosen based on secondary sexual characteristics. These characteristics were large, muscular pads on the dorsal surface of

the head, darkened coloration, well-developed genital papilla, and indications of possible aggressive tendencies toward other males as evidenced by the presence of wounds in and around the head region (Bart et al. 1998; Dunham et al. 1999; Kristanto 2004). These wounds on males during the breeding season are indicative of territoriality among males in a pond.

A total of six spawning trials were conducted over a period of 4 weeks during the 2004 spawning season. Two spawning trials occurred during the week of May 23-29, 2004 (spawning week 1). A single spawning trial occurred during the week of May 30-June 5, 2004 (spawning week 2). During the final spawning week (spawning week 3; June 13-19, 2004), three spawning trials were conducted and all remaining fish were removed from each brood pond. Those fish that still did not exhibit well developed secondary sexual characteristics were culled and were considered not gravid that would not spawn during the current spawning season.

For the 2005 spawning season, one spawning trial was conducted. All females were removed from the ponds and implanted with LHRHa.

Preparation and Administration of Hormone Solution

LHRHa was obtained from American Peptide Company in a fine powder form. Its chemical name is des-Gly^{10},[D-Ala^{6}]LH-RH Ethylamide. Its amino acid profile is pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NHEt. A stock solution of LHRHa was made by diluting 1 mg of LHRH in 1 mL of commercial 0.85% physiological saline solution for a stock solution of 1,000 ug/mL. For priming doses, this solution was further diluted to form a stock solution of 200 ug/mL.

Before injection, each fish was weighed and individually bagged in mesh bags. Each bag was labeled with the fish's weight and individual fish number. This fish number was used to track individual fish and subsequent egg masses. Once the bagged fish were labeled, the bags were clipped to the side of holding tanks using clothes pins. These holding tanks were approximately 3.0m X 0.47m X 0.61m in dimension with a capacity of between 670 and 837 liters of water. Each holding tank was supplied with continuous flow through water and air stones supplying oxygen. Flow rates were maintained at an average rate of 32.8 L/minute, and dissolved oxygen concentration in the tanks averaged 7.4 mg/L. A maximum of 10 bagged fish were placed in each holding tank. The main purpose for limiting only ten fish per holding tank was to allow adequate space between bags so that entanglement among bags did not occur.

In 2004, fish were intraperitoneally injected with a priming dose of 20 ug LHRHa/kg of fish body weight. Twelve hours later, a resolving dose of 100 ug LHRHa/kg of fish body weight was intraperitoneally injected (LHRHa powder was 82% active ingredient). A standard 21 gauge hypodermic needle was used to perform all injections. Fish were injected just posterior to the pelvic fins.

In 2005, females were placed in 120L aquaria once the ovulating agent had been administered. The hormone dosage was administered by inserting a 100 ug LHRHa implant (Mylonas and Zohar 2001). The implant was placed approximately 3 cm posterior to the dorsal fin and 4 cm ventral to the top of the fish. A standard 12 gauge hypodermic needle was used to insert the implant.

Sperm Preparation

Blue catfish males were sacrificed no more than 24 hours prior to use. The testes of these sacrificed males were removed from the abdominal cavity, taking care to remove the testes in a manner that allowed a minimal amount of mesenteric tissue to remain attached to the extracted testicular tissue. As the testes were removed, they were transferred to a plastic dish, and, if needed, rinsed with saline solution (8.5g of pickling salt/1 L of distilled water) to remove excess blood. The testes were examined and only good quality testicular material was kept to fertilize eggs. Testes of good quality had a whitish coloration and that appeared to be full of milt. Sections of testes that were reddish in coloration and that did not appear to be full of milt was subsequently removed and discarded. The remaining testicular material was trimmed, removing any extra mesenteric tissue that remained attached.

Once the testes were trimmed, the weight of the testes was determined. The testes were then placed in a sealed plastic bag so that they could be macerated to release the milt. Maceration was accomplished manually or by mashing with a wooden block. To aid the separation of the milt from the testicular material small amounts of saline solution were added as long as the total amount of saline used did not exceed 10ml of saline per gram of testes.

In 2004, the resulting saline/sperm solution was strained through a fine mesh strainer and the remaining tissue was discarded. The bag that was used to macerate the testes was rinsed with another small amount of saline solution not letting the total amount of saline used exceed 10mL per gram of testicular tissue. This solution was strained and added to the first decantation. At this time, enough saline was added so that the final concentration of sperm solution was 10mL of saline per gram of testes macerated.

In 2005, the sperm preparation procedure slightly deviated from that used in 2004. Once enough saline solution was added to bring the concentration too 10ml of saline per gram of testes macerated, the solution was then analyzed using a spectrophotometer to determine the concentration of sperm. Once the concentration of the sperm was determined, it could then be further diluted so that approximately 6.5 X 10^7 sperm per 100 eggs was used.

Artificial Spawning

Beginning approximately 3 hours prior to the time when ovulation was expected, females were periodically checked to see if any eggs had been released as indicated by eggs attached to the mesh bags. When a female was found ovulating, she was removed from the holding tank still contained in the mesh bag, and placed in a 200 ppm tricaine methane sulfate (MS-222) solution. To buffer the pH of the anesthetic, 200 ppm of sodium bicarbonate was also added to the tub containing MS-222. The ovulating females were submerged in the solution until the opercula movement slowed. Once opercula movement slowed, the female was removed from the mesh bag and then from the MS-222 solution, and rinsed in freshwater. Females were gently dried and the head wrapped inside a towel and moved to the stripping table. During this entire period, whenever a fish was held out of the water care was taken to plug the vent with a finger to prevent the premature release of eggs.

Eggs were stripped into a dried aluminum pie pan, which had been lightly coated with vegetable shortening. Approximately, 200g of eggs were added to each pie pan until the female failed to express eggs. When blood was expressed, the eggs were rinsed with a saline solution (8.5g of salt/1 L of distilled water) to remove the blood. If clumped

eggs or eggs connected by ovarian tissue were stripped, these clumps were manually removed. Each pan of eggs was weighed to the nearest gram to determine the approximate number of eggs in the pan.

Fertilization and Incubation

In 2004, fertilization of eggs was accomplished by adding 2.5mL of the sperm solution for every 100g of eggs. The sperm from different males were combined into one stock solution, so eggs from each female were fertilized by two or more males. Once sperm solution had been added to a pan of eggs, water was added to activate the sperm-egg mixture for fertilization. The eggs were gently swirled, for 1-2 minutes, until thoroughly mixed.

After fertilization, the eggs were allowed to sit undisturbed for at least two minutes to allow the eggs to begin to form an egg mass. Subsequently, the eggs were transferred to a trough filled with pond water for further water hardening. Water hardening was allowed to continue for at least fifteen minutes. After water hardening, the egg masses were transferred to plastic mesh baskets in paddle wheel troughs for incubation. Their position in each trough was recorded and the individual location was labeled. This was done to ensure that each individual egg mass could be traced back to the correct female for data analysis.

Once placed in the paddle wheel troughs, the eggs were incubated until hatch. Approximately 24 hours after the eggs were placed in an individual trough, formalin (100 ppm) and copper sulfate (32 ppm) treatments began to try and control fungus growth. Formalin treatments were always applied first. The eggs were treated a total of three times a day at approximate 8 hour intervals with two treatments of copper sulfate

(morning and late evening) and one treatment of formalin (afternoon). Treatments continued until the first fry began to hatch in the trough. If fungus became prevalent on an individual egg mass, fungus was removed, or if the fungus became severe enough, the entire egg mass was removed.

In 2005, the treatment of eggs deviated slightly from that used in 2004. Based on data from Small and Chatakondi (2006) that illustrated that the fertilization rate and survival of fry could be greatly improved if the eggs were not treated with formalin or copper sulfate during a particular critical period of time after fertilization, the eggs during 2005 were not treated between 42 and 46 hours after fertilization when the water was 28^{o}C. By converting this time to temperature hours, the critical period is 1,176-1,288 temperature -hours. However, once this critical period elapsed, eggs were once again treated approximately every eight hours, using copper sulfate in the early morning and late evening and formalin in the afternoon. Eggs were treated until the first fry began to hatch in the trough.

Data Analysis

Spawning traits measured included the percent females culled (number of females culled/total number of females), ovulation rate (number ovulating females/total number of females injected), spawning rate (number ovulating females/total number of females in strain), latency period (the time from the priming does of LHRHa until the fish ovulated), and relative fecundity (total number of eggs spawned by a female divided by the female body weight).

Egg quality characteristics were subjectively described as good (bright yellow coloration), whiteness, bloodiness, clumpiness, or presence of excess fluid. Subjective

evaluations were made for each pan of eggs was rated on a 1 to 5 scale. A score of 5 was assigned for eggs that were deemed of good quality with yellow color that did not have any blood. Eggs that flowed freely but were sticky and had a pale yellow color were assigned a score of 4 while a score of 3 was assigned to free flowing eggs that contained clumps and some blood. A score of 2 was assigned to free flowing eggs that contained clumps, blood and extra fluid while eggs that were white with excessive blood, clumps and extra fluid were assigned a score of 1 (Kristanto 2004).

The hatching percentage of individual egg masses was estimated visually approximately 6-12 hours prior to expected hatch of fry. At this time fry were visibly motile and vigorously spinning in the egg casing making it possible to estimate the percentage of fry alive in the entire egg mass. Each individual egg mass was weighed. Total number of fry alive was estimated by multiplying the percent alive of an individual egg mass by the total egg mass weight by 40 eggs per gram (Phelps). Hatch percentage was then calculated as the total number of fry produced by an individual female divided by the fecundity of that female. Fry per kilogram of female body weight was calculated as the total number of fry produced by a genotype divided by the body weight of all fish injected belonging to that genotype.

Statistical analyses were performed using either Statistical Analysis Software version 9.1 or Microsoft Excel 2003. ANOVA was conducted followed by Duncan's Multiple Range Test to compare means among genotypes. Pearson chi-squared goodness of fit test was used to analyze the percentage of females culled, the ovulation rate, and spawning percentage of the various strains.

RESULTS:

2004 Spawning Season

Culled Percentage, Ovulation Percentage, and Spawning Percentage

For percent culled, there were no differences among the following select lines and their corresponding randomly bred control, AU-1 and AU-1 control (P=0.2094), AU-7 and AU-7 control (P=0.7860), AU-3 and AU-7 control (P=0.5995), and AU-6 and AU-7 control (P=0.9152; Table 14). However, differences were observed between the selected line AU-4 (86.1%) and its randomly bred control AU-1 control +AU-7 control (68.2%; P=0.0031; Table 14). Significant differences were also observed between the selected line AU-8 (89.5%) and its randomly bred control AU-1 control +AU-7 control (68.2%; P=0.0403; Table 14).

For ovulation percentage, no significant difference existed between any selected line and its randomly bred control (Table 14). For the selected line AU-1 and its randomly bred control AU-1 Control (P=0.1452), each had ovulation percentages in excess of 60.0% (Table 14). For selected line AU-7 and AU-7 Control (P=0.8002), both had an ovulation percentage in excess of 80% (Table 14). Ovulation percentage was also in excess of 80% for the selected line AU-6 versus its randomly bred control AU-7 Control (P=0.3450), the selected line AU-4 and its randomly bred control AU-1Control+AU-7 Control (P=0.5082), and the selected line AU-8 versus its randomly bred control AU-1 Control+AU-7 Control (P=0.2023; Table 14). For the remaining

Table 14: % of channel catfish, *Ictalurus punctatus*, females gravid, culled, ovulating, and spawned for each selected strain compared to its randomly bred control during the 2004 spawning season when induced to spawn with 20 and 100 μg/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog. Each strain couplet represents a different selected line (top) compared to its randomly bred control (bottom).

Strain	N	% Gravid	% Culled	% Ovulation	% Spawned
AU-1	35	74.3	25.7	61.5	45.7
AU-1 Control	60	61.7	38.3	78.4	48.3
AU-7	24	79.2	20.8	84.2	66.7
AU-7 Control	28	82.1	17.9	87.0	71.4
AU-3	52	86.5	13.5	77.8	67.3
AU-7 Control	28	82.1	17.9	87.0	71.4
AU-6	21	81.0	19.1	94.1	76.2
AU-7 Control	28	82.1	17.9	87.0	71.4
AU-4	36	86.1*	13.9*	87.1	75.0*
AU-1C+AU-7C	88	68.2*	31.8*	81.7	55.7*
AU-8	57	89.5*	10.5*	90.2	80.7*
AU-1C+AU-7C	88	68.2*	31.8*	81.7	55.7*

*indicates that there are significant differences between the couplet.

comparison, AU-3 versus AU-7 Control (P=0.3625), both had ovulation percentages in excess of 75% (Table 14).

No significant differences between the following genotypes existed for spawning percentage: AU-1 and AU-1 control (P=0.8052), AU-7 and AU-7 control (P=0.7107), AU-3 and AU-7 control (P=0.7045), and AU-6 and AU-7 control (P=0.7087; Table 14). However, significant differences were seen between the selected line AU-4 (75.0%) and its randomly bred control AU-1 control +AU-7 control (55.7%; P=0.0020; Table 14) and a significant difference was seen between the selected line AU-8 (80.7%) and its randomly bred control AU-1 control +AU-7 control (55.7%; P=0.0450; Table 14).

Latency Period

Pooled latency periods for all spawning weeks were not different (P>0.05) between all selected lines and their corresponding controls except for only one selected line, AU-8, that exhibited a shorter latency period compared to its randomly bred control (P=0.0337; Table 15). AU-8 had a mean latency period 1.2 hours shorter than AU-1 control + AU-7 control (Table 15). No genotype x spawning week interaction was evident in any of the comparisons (P>0.05; Table 15).

However, significant effects of spawning week (P<0.05) on mean latency period were observed. However, there was no difference (P>0.05) in latency period for any of the selected lines and their corresponding randomly bred controls regardless of week (Table 15). Thus, because there was a significant effect of spawning week, and differences between selected lines and randomly bred controls were not seen in any individual week, then the significant difference between AU-8 and AU-1 control + AU-7

Table 15: Mean latency period for selected strains and their randomly bred controls that were spawned during all weeks combined, and each individual spawning week. Mean water temperatures during individual spawning weeks were: week 1 was 27.0°C, week 2 was 28.4°C, and week 3 was 27.8°C. Each strain couplet represents a different selected line (top) compared to its randomly bred control (bottom).

	Week 1		Week 2		Week 3		All Weeks	
Strain	**N**	**Mean +/- SD**	**N**	**Mean +/- SD**	**N**	**Mean +/- SD**	**N**	**Mean +/- SD**
AU-1	14	42.3 ± 2.2	1	48.8 ± 0.0	2	41.1 ± 1.6	17	42.5 ± 2.6
AU-1 Control	13	42.0 ± 2.7	5	50.7 ± 2.2	13	40.5 ± 2.5	31	42.8 ± 4.4
AU-7	10	42.4 ± 1.7	2	50.9 ± 3.5	3	43.2 ± 2.0	15	43.7 ± 3.5
AU-7 Control	6	42.7 ± 2.2	2	50.4 ± 2.6	12	40.9 ± 3.6	20	42.4 ± 4.2
AU-3	12	42.3 ± 2.9	6	49.8 ± 2.2	17	39.3 ± 3.1	35	42.1 ± 4.7
AU-7 Control	6	42.7 ± 2.2	2	50.4 ± 2.6	12	40.9 ± 3.6	20	42.4 ± 4.2
AU-6	7	40.8 ± 1.9	1	48.0 ± 0.0	8	40.9 ± 2.9	16	41.3 ± 2.9
AU-7 Control	6	42.7 ± 2.2	2	50.4 ± 2.6	12	40.9 ± 3.6	20	42.4 ± 4.2
AU-4	15	41.7 ± 2.2	4	49.5 ± 1.7	8	41.2 ± 3.5	27	42.7 ± 3.8
AU-1C+AU-7C	19	42.2 ± 2.5	7	50.6 ± 2.1	25	40.7 ± 3.0	51	42.6 ± 4.3
AU-8	17	41.3 ± 2.4	5	49.4 ± 1.5	26	39.9 ± 3.6	48	41.4 ± 4.1*
AU-1C+AU-7C	19	42.2 ± 2.5	7	50.6 ± 2.1	25	40.7 ± 3.0	51	42.6 ± 4.3*

*—indicates that there are significant differences between the couplet.

control was probably an artifact due to random differences in individuals that were spawned during each week.

Egg Quality

Pooled egg quality scores for a selected line compared to its randomly bred control for all spawning weeks were not different (P>0.05) for any pair (Table 16). All genotypes had mean egg quality scores in excess of 3.5 but equal to or less than 4.0 (Table 16). Furthermore, for any of the pairings, there was not a significant effect (P>0.05) of spawning week on mean egg quality scores. No (P>0.05) genotype x spawning week interaction existed for all except one pair (AU-1 vs. AU-1 control; P=0.0104).

Relative Fecundity

No effect of spawning week (P>0.05) or genotype x spawning week interaction (P>0.05) was observed with regards to mean relative fecundity for each of the selected line versus randomly bred controls. However, there was a single pair (AU-8 and AU-1 control + AU-7 control) for which there was a significant difference (P=0.0184). The selected line AU-8 had a significantly greater relative fecundity rate (10,202 eggs/kg) than the control AU-1 control + AU-7 control (8,543 eggs/kg; Table 16). Additionally, for the comparison of the selected line AU-1 and the control AU-1 control, no differences (P=0.3986) were observed when the data from 2004 and 2005 were pooled.

Hatch Percentage

For each selected line and its randomly bred control, there was no effect of spawning week (P>0.05) or genotype x spawning week interaction (P>0.05) on mean hatch percentage. Also, there was only a single pair (AU-6 and AU-7 control) for which

Table 16: Mean egg quality scores, fecundity, hatch %, and fry/kg for selected channel catfish female strains and their randomly bred controls that were spawned during all weeks combined, and each individual spawning week. Each strain couplet represents a different selected line (top) compared to its randomly bred control (bottom).

	Egg Quality		Fecundity (Eggs/kg)		Hatch %		Fry/kg	
Genotye	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD
AU-1	13	4.0 ± 0.9	16	6,575 ± 2,863	16	33.5 ± 24.8	16	2,173 ± 1,739
AU-1 Control	29	4.0 ± 0.6	29	8,138 ± 3,275	29	36.7 ± 34.9	29	3,066 ± 3,141
AU-7	16	3.9 ± 0.8	16	8,150 ± 2,617	16	10.8 ± 9.8	16	801 ± 793
AU-7 Control	18	3.6 ± 1.1	20	9,130 ± 3,922	20	10.9 ± 15.1	20	993 ± 1,169
AU-3	32	3.9 ± 0.6	35	9,207 ± 3,490	35	30.4 ± 44.9	35	2,313 ± 2,473*
AU-7 Control	18	3.6 ± 1.1	20	9,130 ± 3,922	20	10.9 ± 15.1	20	993 ± 1,169*
AU-6	16	3.8 ± 0.5	16	9,479 ± 2,398	16	29.2 ± 24.5*	16	2,849 ± 2,670*
AU-7 Control	18	3.6 ± 1.1	20	9,130 ± 3,922	20	10.9 ± 15.1*	20	993 ± 1,169*
AU-4	25	3.8 ± 1.0	27	8,841 ± 3,273	27	22.5 ± 28.2	27	1,870 ± 2,067
AU-1C+AU-7C	47	3.8 ± 0.9	49	8,543 ± 3,548	49	26.1 ± 31.0	49	2,220 ± 2,712
AU-8	45	3.7 ± 0.8	46	10,202 ± 3,120*	46	22.9 ± 35.0	46	2,124 ± 2,400
AU-1C+AU-7C	47	3.8 ± 0.9	49	8,543 ± 3,548*	49	26.1 ± 31.0	49	2,220 ± 2,712

*indicates that there are significant differences between the couplet.

there was a difference (P=0.0127) for hatch percentage. Again, the selected line AU-6 (29.2%) had a greater hatch percentages than the control AU-7 control (10.9%; Table 16). For the comparison of the selected line AU-1 versus the control AU-1 control, no differences (P=0.1557) between genotyes were also observed when the data from 2004 and 2005 were pooled.

Fry/Kg

No effect (P>0.05) of genotype x spawning week interaction existed for any of the selected lines versus randomly bred control comparisons. The selected line AU-3 (2,313 fry/kg) produced more fry/kg than the control AU-7 control (993 fry/kg; Table 16) when data from females spawned during all spawning weeks is pooled. Also, for the above pairing, a spawning week effect (P=0.0158) on fry/kg production was also evident.

During spawning week one (May 23-29, 2004; P=0.3741) and spawning week three (June 13-19, 2004; P=0.2197) there was no effect of genotype on fry/kg production when comparing AU-3 to AU-7 control (Table 17). But, during spawning week two (May 30-June 5, 2004) the selected line AU-3 (5,051 fry/kg) produced more fry/kg than the control genotype AU-7 control (2,386 fry/kg; P=0.0429; Table 17).

Also a significant genetic effect existed when comparing the selected line AU-6 (2,849 fry/kg) to AU-7 control (993 fry/kg; P=0.0114) for fry/kg production when fish spawned during all spawning weeks are compared (Table 16). Once again, the selected line produced more fry/kg than the randomly bred control. For all other selected lines versus their randomly bred control genotypes, there was no effect (P>0.05) of genotype or spawning week on fry/kg production. For the comparison of the selected line AU-1

Table 17: Mean fry/kg comparison for strain AU-3 versus AU-7 Control. Couplet represents a different selected line (top) compared to its randomly bred control (bottom).

	Week 1		Week 2		Week 3		All Weeks	
Genotype	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD
AU-3	12	1,629 ± 2,089	6	5,051 ± 1,381*	17	1,820 ± 2,432	35	2,313 ± 2,473
AU-7 Control	6	813 ± 771	2	2,386 ± 670*	12	851 ± 1,288	20	993 ± 1,169

*—indicates that there are significant differences between the couplet.

versus the control AU-1 control, no differences (P=0.2306) were observed when the data from 2004 and 2005 were pooled.

2005 Spawning Season

During the 2005 spawning season when the selected line AU-1 was compared to the randomly bred control AU-1 control as four year-olds, no difference (P=0.6410) in ovulation percentage was observed between the two genotypes (Table 18). Also, no difference in latency period (P=0.3399), egg quality (P=0.2666), relative fecundity (P=0.8806), hatch percentage (P=0.2584), or fry/kg female (P=0.3535; Table 18) existed among genotypes.

Table 18: Ovulation %, mean latency period, mean egg quality score, mean hatch %, mean fecundity (eggs/kg), and mean fry/kg for strains spawned during the 2005 spawning season using a 100ug implant of luetinizing hormone-releasing hormone analog (LHRHa). Couplet represents a different selected line (top) compared to its randomly bred control (bottom).

	Ovulation %		Latency		Egg Quality		Fecundity (Eggs/Kg)		Hatch %		Fry/Kg	
Genotype	N	%	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD	N	Mean +/- SD
AU-1	13	69.2	9	73.4 ± 9.5	2	5.0 ± 0.0	9	10,581 ± 2,366	9	32.5 ± 31.4	9	3,772 ± 3,443
AU-1 Control	18	61.1	10	68.8 ± 10.9	6	4.5 ± 0.5	11	10,360 ± 3,776	11	58.2 ± 59.5	11	5,552 ± 4,650

*indicates that there are significant differences between the couplet.

DISCUSSION:

Most channel catfish lines that had been selected for faster growth rates exhibited a similar culling percentage, ovulation percentage, and spawning percentage as the randomly bred control. For all genotypes analyzed over both spawning seasons, ovulation percentage was in excess of 60%. There were two exceptions to this generality. The selected lines AU-4 and AU-8 exhibited lower culling percentages and greater spawning percentages than the control AU-1 control + AU-7 control.

This suggests that inbreeding depression, which can become a problem in mass selected lines, is not causing a reduction in these reproductive traits. In some cases, a correlated response between direct selection for body weight and reproductive performance may be occurring.

Mean egg quality scores of channel catfish female lines that had been selected for faster growth were not different from their respective randomly bred controls, during 2004 and 2005. Egg quality scores of all genotypes were high with a consistent quality and characterized as free flowing with a pale yellow coloration with very few clumps or bloodiness.

Selection for body weight usually did not affect fecundity of females. There was one exception to this generality. When the selected line AU-8 was compared to the randomly bred control AU-1 control + AU-7 control, the selected line produced approximately 1,700 more eggs per kilogram of female body weight than the control. A

positive correlated response for fecundity was evident. Dunham and Smitherman (1984) observed a similar correlated response for fecundity when selecting for body weight when looking at Rio Grande and Marion genotypes.

Furthermore, the lack of any depressed fecundities in the selected lines in comparison to the controls suggests that inbreeding depression has not affected this reproductive trait. Several researchers had found that inbreeding depression has caused a reduction in fecundity in other fish species (Su et al. 1996; Heath et al. 2002; Gallardo et al. 2004). Thus, since no inbreeding depression was evident for fecundity, it can be concluded that the level of inbreeding in the selected lines has not yet become a problem.

The same general trends exhibited for relative fecundity were observed for both hatch percentage and fry production. For most of the comparisons examined, there appears to be no difference in hatch percentage or fry production between the selected line and their respective control. For most genotypes selected for increased body weight, there was no negative correlated response or inbreeding depression for hatch percentage and hybrid fry production.

For hatch percentage, there was one exception to the above generality. When the hatch percentage of the selected line AU-6 was compared to the control AU-7 Control, the selected line exhibits a 20% increase in hatch percentage compared to the control. This is a possible indication of a positive correlated response between selection for body weight and CB hybrid hatch.

All genotypes analyzed over both spawning seasons exhibited a range of values between 1,000 and 5,600 fry/kg. CB hybrid fry production of all genotypes was greater than that observed by Dunham et al. (2000) when producing hybrids using CPE as the ovulation agent and equal to or greater than the fry production per kilogram of female body weight when channel catfish females were spawned with channel catfish males. Fry production also fell within the range of CB hybrid fry production observed by Kristanto (2004).

There were two exceptions, with regards to CB hybrid fry production, to the generality that the selected lines exhibit statistically similar fry production as randomly bred controls. First, when the selected line AU-3 was compared to its control AU-7 Control the selected line produced 1,300 more fry/kg of female body weight than the control, thus suggesting a possible positive correlated response between selection for body weight and CB hybrid fry production. The second exception was when comparing the selected line AU-6 to the control AU-7 Control. In this case, the selected line produced approximately 1,900 more fry/kg of female body weight than the control. Once again, this suggests a possible positive correlation between direct selection for body weight and CB hybrid fry production.

The effect of inbreeding depression on hatching percentage and fry production has been consistent in fish. Kincaid (1976a) and Su et al. (1996) reported that there was a reduction in hatching percentage and fry survival with inbred rainbow trout. However, other researchers have reported that inbreeding depression has caused a reduction in

hatch and fry survival in zebra fish (Markovic and Haley 1979), channel catfish (Bondari and Dunham 1987), rainbow trout (Kincaid 1976a; Kincaid 1976b), coho salmon (Gallardo et al. 2004), and Chinook salmon (Heath et al. 2002). Inbreeding depression has not started affecting reproductive traits in the selected channel catfish female lines.

Variable results were obtained for the effect selection for increased body weight for 6-7 generations on reproductive traits of channel catfish females. In two cases, where lines were developed from multiple strains, AU-4 and AU-8, there appears to be no decrease in reproductive traits after 6-7 generations of selection, and no inbreeding depression. In a third case, AU-3, involving a line developed from multiple strains, there appears to be a positive correlated response between direct selection for body weight and reproductive traits. However, the difference between the select lines and the controls could be an age effect, since the only controls available were three-years-old and the selected individuals were four-years-old.

AU-1, AU-6 and AU-7 were developed in each case from a single strain with AU-1 derived from a single river collection and AU-6 and AU-7 closely related and likely originating from multiple rivers. AU- 6 also appeared to exhibit a positive correlated response for fry production when selecting for growth rate. Again, this may have been due to an age effect or inflated because despite a similar history AU-7 appears to have consistently poor performance.

In the case of AU-7, there was no apparent correlated response or inbreeding depression, and the fish were all of the same age. AU-7 consistently performed more

poorly than its control, and either exhibits a negative correlated response, inbreeding depression or both.

The adverse effects of mass selection for body weight on reproductive performance may be more severe than what these results indicate. Smitherman and Dunham (1985) indicate that in early generations of individual selection for body weight positive correlated responses occurred for reproductive traits. In the cases, where the select lines are not different from controls or have decreased means compared to controls, there decrease in performance is even greater if they once had higher means than randomly bred controls.

Selection for increased body weight appears to have variable effects on reproduction and hybrid fry production when channel catfish females are induced to ovulate with LHRHa, and are strip spawned. Increased, decreased or no change in reproductive output was observed. This variable result may have been partially caused by the genetic diversity of the founder population of the select line, however additional experimentation is needed to confirm this hypothesis. The variable results prevent making a specific recommendation on the use of mass selected lines for body weight for production of hybrid fry. However, breeding history must be considered as utilizing fish from a narrow genetic base did have an adverse effect on channel-blue hybrid fry production.

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