IMPROVING REPRODUCTIVE EFFICIENCY TO PRODUCE CHANNEL × BLUE HYBRID CATFISH FRY

Reporting Period
March 1, 2004 - August 31, 2007

Funding Level	Year 1	$118,390
	Year 2	$111,610
	Year 3	$115,000
	Year 4	$115,000
	Total	$460,000

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PROJECT OBJECTIVES

1. Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.
    a. Determine minimum cold period required and rates and patterns of application of thermal changes to promote synchronous gonadal development and spawning.
    b. Improve hybrid embryo production by determining the best nutritional regime to maximize fecundity and hatch rate from induced channel catfish females and blue catfish males.
    c. Improve hybrid embryo production via genetic enhancement.
2. Develop induced spawning techniques and management strategies to optimize gamete collection and storage.
    a. Develop procedures to predict ovulation of channel catfish.
    b. Conduct pivotal protocol studies for determining dosage rates and timing of application of luteinizing hormone releasing hormone (LHRHa), carp pituitary extract and catfish pituitary extract to maximize ovulation, hatch rate and fry production.

c. Improve hybrid embryo production via pheromonal manipulation of channel catfish males and blue catfish males for improved ovulation, spermiation, egg quality, hatch and fry production.
d. Develop extended refrigerated storage and cryopreservation of sperm.
e. Develop short-term extended storage of eggs.

3. Develop techniques to identify, assess and improve gamete quality.

a. Develop criteria for standardizing and classifying egg quality prior to injection and after manual stripping and describe the morphological and physiological condition of channel catfish eggs including evaluation of morphological changes of oocytes during oocyte maturation in female catfish.
b. Determine the profile of estradiol hormone from serum plasma of 2-year-old female channel catfish over a 12-month period, determine changes in oocyte maturation during vitellogenesis and identify the different cathepsins that are responsible for vitellogenin degradation and oocyte maturation in female catfish.
c. Develop in vitro assays to evaluate sperm quality and evaluate their predictive ability in relation to fertilization and hatch.

4. Develop economically viable standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.

a. Determine optimal sperm (fresh, frozen and refrigerated)-to-egg ratios for fertilization and hatch.
b. Determine the effects of commonly used therapeutics on hatching success.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.*

Objective 1a. *Determine minimum cold period required and rates and patterns of application of thermal changes to promote synchronous gonadal development and spawning.*

Louisiana State University and University of Memphis. Water temperature is the primary environmental factor affecting the spawning of channel catfish. Spawning begins when water temperatures consistently remain above 21°C at some locations such as Louisiana and west Mississippi. The spawning season at the Aquaculture Research Station of the Louisiana State University Agricultural Center was lengthened by heating ponds through addition of geothermal water (36°C). This study attempted to use degree-days to describe and quantify the total heat requirement for channel catfish to initiate spawning, which should also indicate the same requirement to initiate artificial spawning to produce hybrid embryos. Degree-days were calculated for 153 spawns between 1999 and 2004. Ponds from 1999 to 2002 had four available spawning sites (cans), and in 2003 and 2004 the

ponds had six sites. Degree-days needed to obtain the first four (1999-2002) or six (2003-2004) spawns were calculated to prevent spawning site limitations effects on the degree-day values.

In 2004, three heated ponds were maintained at three different temperatures. Degree-day values were calculated for 18 spawns using three threshold temperatures as the starting point to calculate the degree-days (Table 1). The 21°C threshold yielded a constant value of 98 ± 4 degree-days for the heat requirement of channel catfish to initiate spawning.

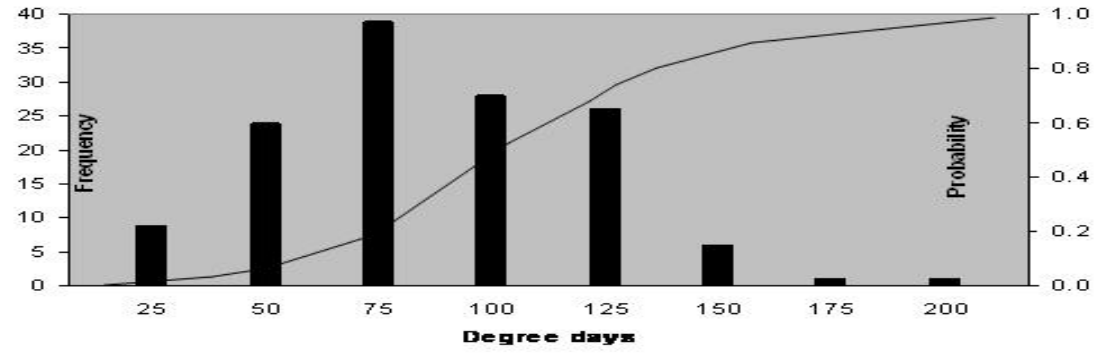
Degree-days were also calculated using the 21°C threshold for 135 spawns collected during the early spawning and regular spawning periods between 1999 and 2003. The average degree-day value above the 21°C threshold was 97 ± 33 degree-days. Spawning probabilities and frequency of spawns were plotted against degree-day values (Figure 1). The probability that a fish will spawn after 100 degree-days was 50% and increased to 93% after 150 degree-days. Fifty percent of spawns occur between 75 degree-days and 125 degree-days and ninety percent between 50 degree-days and 150 degree-days. These results concur with the literature that 21°C is the minimal water temperature needed to initiate the reproductive process in channel catfish.

Additionally, degree-days above 21°C may be useful as a management tool to predict channel catfish spawning times in heated ponds, and the correct time to initiate artificial spawning for hybrid embryo production.

Table 1. The average degree day value for spawns above three thresholds from ponds maintained at different temperatures. Values in the same column followed by the same letter do not differ significantly ($P < 0.05$).

		Threshold		
Target temperature	Actual temperature	18°C	21°C	24°C
21°C	23.1 ± 1.5°C	234 a	95 a	8 a
24°C	23.1 ± 2.6°C	203 ab	98 a	22 b
27°C	24.6 ± 3.0°C	184 b	102 a	41 c

Figure 1. Spawning probabilities and spawning frequency at different degree-day values above the 21°C threshold.



Results at a glance...

- *A water temperature of 21°C is the minimal temperature needed to initiate the reproductive process in channel catfish. Tracking degree-days above 21°C may be useful as a management tool to predict channel catfish spawning times and will be especially useful as a tool to predict best times to initiate artificial spawning for hybrid embryo production.*

In 2005, the temperature data results from previous years was used to compare reproductive performance of channel catfish females induced to spawn before and during the natural spawning season. The goal was to extend the documented time female channel catfish could be induced to spawn before the natural spawning season without affecting reproductive performance.

In December of 2004, channel catfish brood stock (1.17 ± 0.38 kg; 48.1 ± 4 cm) were purchased from Haring Fish Farms, Inc., a commercial fingerling producer in northern Louisiana. Broodfish were stocked in nine, 0.04-ha earthen ponds at the Aquaculture Research Station of the LSU Agricultural Center. Six of the ponds were stocked with 30 females and 10 males. The remaining ponds were stocked with 30 females only. Geothermal water (36°C) was added to three of the ponds (two mixed-sex and one all-female) beginning on January 13, 2005. Initial pond temperatures were approximately 20°C and were increased 2°C/day until the temperature reached 28°C. Ponds were heated in sets of three until natural spawning occurred. After six spawns (egg masses, 20% spawning) were collected from spawning containers in the ponds, fish were collected by seining and brought indoors for induced spawning.

Broodfish were acclimated in an indoor recirculating system for 48 to 72 hours. Females were evaluated using ultrasound imaging of ovaries and oocytes to assess reproductive readiness. Selected females were weighed, measured, marked for identification, and placed into eight, 120-L fiberglass tanks. Pairings were made based on length and weight to minimize size differences within the tanks. Four tanks held one male and one female (mixed-sex pairs); the other four tanks contained two female fish (female pairs). Each female was given a single injection of leutenizing hormone-releasing hormone analog at a dosage of 100 μg/kg. Temperature was maintained at 27°C and spawning behavior was monitored every 2 hours. After females began releasing eggs they were anesthetized (tricaine methane sulphonate, MS-222) and manually stripped.

There was no significant difference in total number of eggs produced per female or in latency (time from hormone injection to spawning) between fish spawned in 15 heated ponds before the natural spawning season and fish spawned in 27 unheated ponds during the natural spawning season. Fertilization for eggs from females from heated (35%) and unheated (43%) ponds were similar. Likewise, hatching rate for eggs from heated (38%) and unheated (34%) females did not differ. There was no significant difference in number of eggs produced per female or latency between fish induced to spawn 63, 47, and 12 days before the natural spawning season. This study showed that channel catfish females can be induced to spawn as early as 63 days (02/01/05) before the start of the natural spawning season (4/12/05) without affecting reproductive performance.

Further studies were conducted during 2005-2006 to better characterize the effects of temperature on spawning of female channel catfish before and during the natural spawning season. To determine the patterns of application of thermal changes to promote synchronous gonadal development and spawning, three temperature requirements were evaluated: 1) minimum number of cold degree-days (degree-days calculated for temperatures below

21°C); 2) minimum number of warm degree-days (degree-days calculated for temperatures above 21°C) before the natural spawning season, and 3) minimum number of warm degree-days during the natural spawning season.

In November of 2005, channel catfish brood stock were purchased from Haring Fish Farms, Inc., a commercial fingerling producer in Northern Louisiana. Twelve, 0.10-acre geothermal ponds at the Aquaculture Research Station of the LSU Agricultural Center were stocked with broodfish at a ratio of 4 females to 1 male. The ponds were heated three at a time and temperatures were controlled using geothermal water. Ultrasound was used to classify females during the accumulation of cold degree-days and throughout the subsequent warm degree-days. The first channel catfish to spawn this past research year (by hormonal induction in the laboratory) was recorded on December 24, 2005. In January, pond spawning became possible and after six spawns (egg masses) were collected from spawning containers in the ponds, fish were collected by seining and brought indoors for hormone-induced spawning.

Broodfish were acclimated in an indoor recirculating system for 48 to 72 hours. Females were evaluated using ultrasound imaging of ovaries and oocytes to assess reproductive readiness. Selected females were weighed, measured, marked for identification, and placed into eight 120-L fiberglass tanks. Pairings were made based on length and weight to minimize size differences within the tanks. Four tanks held one male and one female (mixed-sex pairs); the other four tanks contained two female fish (female pairs). Each female was given a single injection of leutenizing hormone-releasing hormone analog at a dosage of 100 μg/kg. Temperature was maintained at 27°C and spawning behavior was monitored every 2 hours. After females began releasing eggs they were anesthetized (tricaine methane sulphonate, MS-222) and manually stripped. All of the spawning and degree-day data for this experiment are being analyzed.

Results at a glance...

- *Early spawning can be accomplished by heating water prior to the natural spawning season without any difference in success compared to the natural spawning season. When 100 degree-hours are reached ovulation and fertilization should be successful. If warm water is available, channel catfish can be successfully spawned in early January.*

Objective 1b. *Improve hybrid embryo production by determining the best nutritional regime to maximize fecundity and hatch rate from induced channel catfish females and blue catfish males.*

Auburn University. Since nutrition can play a key role in maturation, as well as egg and fry quality, proper nutrition could be a key factor in the development of hybrid rearing technologies. Hence, the primary goal of this component of the project is to improve hybrid embryo production through nutrient manipulations.

Protein Level and Feed Frequency

The first subobjective was to evaluate the influence/interaction of dietary protein level and feeding rate on egg production of channel catfish. As there are numerous interactions and it is difficult to obtain brood stock, a holistic approach was initially used to help identify important factors to control.

A total of 495 females were stocked in 16 ponds, using four ponds per treatment. The females were divided in three strains considering previous spawning behavior (high spawning, low spawning, and NWAC103), and based on that characteristic

they were assigned proportionally in a randomized manner to each pond. Fish were pit-tagged and heat-branded for identification. The fish were stocked on February 13, 2004 in 0.04-ha ponds at a density of approximately 1,500 kg/ha, followed by an acclimation period of approximately one month. During the acclimation period, fish were offered a commercial floating feed (32% protein) diet three times a week at 1.5% of their body weight. The two test diets were a 32% typical practical catfish feed and a 42% high fish meal practical catfish feed, and the feed was offered either three or six times a week to apparent satiation. A fifth treatment utilized 32% protein 3 days per week with supplemental feeding of liver two additional days per week at a rate similar to the dry feed. Females were spawned in three periods (early, middle and late spawning periods). Dietary protein level and feeding rate treatments were evaluated using the following indicators: egg mass, number of eggs, fecundity (number of eggs per kilogram female), egg diameter, and fertilization rate 48 hours after fertilization.

Results for this experiment are presented for "High" and "Low" spawning strains, as strain 103 had a low number of individuals per treatment per spawning period. High spawning and low spawning females had a survival of 92% (414 females), of which 63.5% spawned.

Based on logistic analyses by strain, the following results were determined. For strain High, the dietary treatment did not have a significant effect on spawning percentage, but age and spawning period had a significant effect on spawning percentage. For this strain, the odds of females that were 5 years old spawning were 9.4-times higher than females that were 3 years old and 8.4-times higher than females that were 4 years old. The odds of spawning in the early spawning period were 10.6 times higher than for the late spawning period; while the middle spawning period had 5.1-times higher odds of spawning than late spawning period.

Dietary treatment did not have a significant overall effect for strain Low spawning percentage, but when comparisons between treatments were performed, the odds of spawning for treatment 4 (32% protein feed, 3 times/week) were 2.5-times higher than for treatment 3 (32% protein feed, 6 times/week). The other two variables, age and spawning period, had a significant effect on spawning percentage. For this strain, the odds of females that were 5 years old spawning were 5.5-times higher than females 3 years old, and was not different than for 4-year-old females. The odds of spawning in the early spawning period were 13.4-times higher than for the late spawning period; and there was no difference between the middle spawning period and the late spawning period. In general, the two strains exhibited different responses. These results indicate that brood stock management must adjust to strain variations and age.

Although using a holistic approach has disadvantages, we were able to evaluate the effects of strain, age and spawning period on spawning success and egg production. In general, each strain responded differently

Results at a glance...

- *Feeding standard 32% protein floating catfish feed 6 times per week for 2 months prior to spawning gives equal or better fry production compared to high protein diets. Supplementation of broodfish diets with menhaden fish oil, DHA and ARA 2 months prior to spawning can increase hybrid fry output up to 100% if the fish were poorly prepared up to that point of time. If the fish are in excellent condition, the fatty acid supplementation is not necessary. Supplementation of brood stock diets with the feeding of forage fish to channel catfish females, does not greatly impact hybrid fry production, but has strong positive effects on sperm production in blue catfish males.*

with respect to dietary treatments and spawning period. It appears that the high-protein diet when offered 6 times per week resulted in reduced fecundity. With respect to the other treatments there were few differences due to dietary treatments.

Comparison of the response of fish maintained on the 32% protein diet with 3 feedings per week with those that also received liver, there were no notable improvements in egg production. As with the other treatments strain and age influenced the response.

When fry production data for the various ages, strains and spawning period are pooled to represent only the dietary treatments, overall production can be determined. Table 2 presents the combined data for the five dietary treatments for groups of fish that could be followed and that received LHRHa injections (a single 20 μg/kg priming injection and a 100 μg/kg resolving injection). Based on mean separation there were significant differences between treatments with fish maintained on the 32% protein diet offered 6 times per week, producing more fry/kg than those maintained on the 32% protein diet fed 3 times per week with a liver supplement twice a week as well as fish offered the 42% protein diet fed 6 times per week. The treatment with the highest observed mean was 32% protein diet offered 6 times per week although there were no significant differences between fish offered the 32% protein diet 3 or 6 times per week or those offered the 42% protein diet 3 times per week. For unknown reasons, supplementation with liver was detrimental to fry production.

Lipid Source and Ratios (n3:n6)

The second subobjective was to evaluate different ratios of polyunsaturated fatty acids, and their influence on fecundity and on egg quality. A total of 190 females were stocked in eight ponds, using two ponds per treatment. All females were 4-year-old Kansas strain from Harbin Farms in Anthony, Kansas. The fish, which were in relatively poor condition, were stocked on January 7, 2005, giving an acclimation period of approximately 2 months. The trial period was 73 days to 87 days depending on the spawning period. Female brood stock were maintained in 0.04-ha ponds at a density of approximately 600 kg/ha. They were offered a commercial floating feed (32% protein, 5% lipid) diet three times a week at 1.5% of their body weight. Water temperature and dissolved oxygen were measured daily in the early morning and after sunset. After the acclimation period, test diets were offered.

Table 2. Effect of nutrition, (% protein-number of feedings per week) 42-3, 42-6, 32-3, 32-6 and 32-3 plus 2 days of liver, on hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags within tanks. GMP grade LHRHa injections (20 μg/kg priming dose with a 100 μg/kg resolving dose). A mixture of low and high producing lines of channel catfish was utilized. Means followed by different letters are significantly different ($P < 0.05$).

Treatment	N	Fry/kg
32-6	51	1,732 a
42-3	48	1,505 ab
32-3	54	1,476 ab
42-6	54	1,032 b
32-3-2L	54	969 b

The four test diets were based on a commercial catfish feed containing 5% lipid that was top-coated with an additional 2% lipid. Three test diets used a combination of vegetable oil sources proportioning different 18:3n3, 18:2n6 ratios. The lipid sources were mixed in the following ratios: Diet 1 contained soybean oil and linseed oil in a ratio of 0.90:1.00; Diet 2 also contained soybean oil and linseed oil, but in a ratio of 7.00:1.00; Diet 3 contained linseed oil only. The fourth diet was based on fish oil and high DHA and ARA oil sources with an n3:n6 ratio of 3:2. Diet 4 contained menhaden fish oil, high DHA, and high ARA in a ratio of 2:1:1. Thus diets contained a range of n3 and n6 fatty acid combinations (Diets 1-3) as well as one diet (Diet 4) which contained HUFA supplements. The n3:n6 ratios of the oil supplements were approximately: 1:1, 1:4, 4:1, and 3:2, respectively.

Dietary lipid treatments were evaluated using the following indicators: total number of eggs produced, fecundity (number of eggs per kilogram female), and fry production, fry per kg female and overall fry survival. Biochemical analysis will also be conducted on egg samples as indicators of egg quality. Data for treatments evaluating lipid source and different ratios between essential fatty acids (Table 3) is restricted to the first spawning period (early), since there was a hatchery problem for the second spawning period, with a minimum of fertilization across all the treatments. Additionally, one pond of fish evaluated in treatment four during the second spawning period were stressed (due to a broken water-supply line) at their harvest causing around 30% mortality.

Based on initial analyses of the data there are few indications that the dietary treatments had a significant effect on the percentage of fish spawning or the number of eggs produced. However, the various lipid combinations did have a strong effect on fry production. Using lipid supplement with a 4:1 ratio of 18:3n3, 18:2n6 (linseed oil, Diet 3) resulted in very poor fry production. The use of menhaden fish oil with DHA and ArA supplements and a ratio of 3:2 for n3:n6 fatty acids (Diet 4) produced the best results. Fish maintained on this diet produced almost twice the fry as fish maintained on diet supplemented with lipids (primarily18:3n3 and 18:2n6) producing n3:n6 ratios of 1:1 (Diet 1) or 1:4 (Diet 2). These results indicate that highly unsaturated fatty acids, HUFA, are probably a key factor in proper brood stock nutrition.

Lipid Source and Ratios (n3:n6) and Forage Fish

A second evaluation of different ratios of polyunsaturated fatty acids, and their influence on fecundity and on egg quality was conducted in 2006. Additionally, the effect of supplementation with forage fish was examined. A10-week trial was conducted in ponds in Auburn, Alabama. In March, 219 female channel catfish broodfish (Kansas Select from Holland Fish Farm in Mississippi) were stocked into nine, 0.04-ha ponds, for an approximate stocking rate of 1,332 kg/ha. Fish were in good condition

Table 3. Egg and fry production in the early spawning period from broodfish fed four diets containing different lipid sources. Diets are described in the text.

Diet	Female weight (kg)	No. females spawned	No. eggs	Eggs/kg female	No. fry	Fry/kg female	% Hatch
1	1.91	12	198,679	8,680	23,901	1,044	12
2	2.15	10	201,949	9,397	27,841	1,296	14
3	1.86	8	111,294	7,500	7,522	507	7
4	1.64	13	186,056	8,731	47,045	2,208	25

when stocked. Three dietary treatments were randomly assigned and fish were offered feed based on their observed response to floating feeds. Diet 1 was a standard 32% crude protein, 6% lipid floating catfish feed. Diet 2 was the same feed supplemented with forage fish (bluegill and fathead minnows) at approximately 28 kg/ha. Diet 3 was the same catfish feed, but top-coated with 2% lipid (1% menhaden fish oil, 0.5% high-DHA oil and 0.5% high-ARA oil). The DHA and ARA oils contained approximately 40% of the designated HUFA. Biochemical analyses of the feeds indicate that the standard and top-coated diets contained 9.4% and 10.4%, moisture; 32.9% and 32.2% protein, 5.95% and 7.58% fat, 5.06% and 4.64% fiber and 7.05% and 6.74% ash, respectively.

In May, females were harvested and those with good spawning characteristics were selected for hormone injection. Selected females were placed individually in soft mesh bags and transferred to holding tanks supplied with continuous flow-through water. Total length and body weight were recorded. Hormone injections were administered in two doses, a priming injection of 30 μg/kg LHRHa, followed 12 hours later by a resolving dose of 150 μg/kg. Twenty-four hours after the second injection, females were examined for ovulation. Females with released eggs were removed from the holding tank and anesthetized in buffered 250 mg/L tricaine methane sulfonate (MS-222). Females were then stripped and eggs were collected in metal pans previously lubricated with vegetable shortening. Females that did not express eggs were returned then rechecked later. Stripping of gametes ceased when all females had been stripped or attempts to strip them had been made. Eggs were subsampled for later biochemical and visual analyses; the remaining mass was weighed and then fertilized with blue catfish sperm and incubated in paddlewheel troughs. Viable fry were estimated by assessing individual egg masses 24 hours pre-hatch. Pooled fry counts were also determined once the egg masses hatched.

Broodfish fed the top-coated, high lipid diet displayed an increase in percent spawn, general fecundity, mean egg weight, grams of eggs spawned, embryo viability at 24 hours pre-hatch and overall fry production per kg of broodfish stocked (Table 4). The availability of forage fish at this density did not result in any statistically significant effects, however,

Table 4. Summary of induced spawning data for female channel catfish crossed with male blue catfish after being maintained on various dietary treatments over a 10-week period. Values represent the means of three replicate ponds per treatment. Means in the same row with different superscripts are significantly different ($P < 0.05$).

	Standard Feed	Feed and Forage Fish	Fatty Acid Supplemented Feed
kg females/hectare	1358	1460	1457
% spawn	65.2	74.5	77.3
Mean egg weight (mg)	18.9b	19.1b	21.4a
Number of eggs/kg female weight	7435	7141	7266
g eggs/kg female spawned	138.3b	135.3b	155.9a
g eggs/total kg brood fish harvested	87.3	99.8	112.8
Fry/kg brood fish harvested	1,787	2,146	2,370
Fry/kg brood fish that spawned	2,817	2,909	3,392
% viable pre-hatch fry	40.6	41.6	47.4
Total fry	291,117	376,067	433,583

there was a trend of increased spawning percentage, grams of eggs obtained, and increased fry production.

In 2007, two additional studies were conducted to further examine the effects of fatty acid supplementation of brood stock diets on egg quality and production of hybrid embryos by female channel catfish. Two feeding trials were conducted in ponds at Auburn, Alabama. The first trial determined the effects of long term (LT, approximately 12 weeks) and short term (ST, 6 weeks) feeding of a standard catfish feed top-coated with 2% of an enhanced marine oil source. The second trial determined the effects of adding HUFAs derived from fungus and marine algae to a standard catfish feed compared to the standard catfish feed without supplementation. The supplemented feed was prepared by Southfresh Feeds, Inc. The unsupplemented feed was Southfresh's standard catfish feed. Both feeds were fed for 6 weeks to female channel catfish just prior to spawning. Kansas select channel catfish females donated by Leigh Holland of Jubilee Farms in Mississippi were utilized in both experiments.

Fish for the long term/short term experiment were transferred to the research ponds in August, stocked at a density of 23 fish per pond (57.4 kg/pond, 1,418 kg/ha) and maintained on a standard catfish feed offered three times a week (approximate 1.5% body weight per feeding). During the colder months, a small amount of feed was offered once per week when the water was warm enough (> 9°C) for the fish to possibly feed. For the long term treatment (LT) the top-coated feeds (1% menhaden fish oil, 0.5% high-DHA oil and 0.5% high-ARA oil) was offered through the winter (starting in October) until spawning. For the short term treatment (ST) the top-coated feed was only offered for a 6-week period just prior to spawning. The DHA and ARA oils (DSM Nutritional Products, Kaiseraugst, Switzerland) contained approximately 40% of the designated highly unsaturated fatty acid.

The second experiment was designed to evaluate a commercially produced feed using a HUFA supplement (AF). Fish were 5-years-old channel catfish and were received from Jubilee Farms on March 12, held for 2 days in flow through vats, and then transferred to the brood stock ponds and stocked at a density of 24 fish per pond (average 94.8 kg/pond, 2,342 kg/ha). They were offered either a standard diet (SD) or a HUFA-enhanced diet (ED) created by adding HUFA supplement containing a mixture of Aquagrow schizochytrium DHA, Aquagrow ARA, and flax meal which was added as a premix to the Southfresh standard catfish diet at a rate of 1.5%. The supplements were created by fermenting marine algae and fungus.

Females were harvested in May, hormone-induced, and strip spawned. Eggs were fertilized with blue catfish sperm and incubated in flow through paddlewheel troughs for 5-7 days. Grams of eggs per kg fish spawned and the number of fry per kg fish spawned were recorded. When fish were harvesting for spawning, total length (cm) and weight (kg) were measured and relative weight index was calculated for all fish.

There were no statistical differences between treatments in either trial for broodfish length, weight, relative weight index, and any of the spawning characteristics. Furthermore, no difference was observed when fry produced per kg of fish was compared for all four treatments. This data suggests that the costs associated with feeding a high lipid diet in the fall and winter is not justified. Previous research, in which the fish were initially in poor condition, resulted in a strong response to HUFA supplement. The present work does not show a response, possibly indicating that when fish are in good condition, HUFA supplementation may not be required.

Effect of Vitamin C on Reproduction

A control diet variably containing 37 to 200 ppm of vitamin C during the spring feeding season was

compared to brood stock diets containing 500 and 1,000 ppm of vitamin C. Vitamin C levels in the blood and eggs of channel catfish females fed these diets increased with increasing vitamin C in the diet until levels reached 500 ppm (Table 5). Supplementation with 1,000 ppm vitamin C did not elevate tissue vitamin C levels further. However, vitamin C levels continued to increase in liver and testes of channel and blue catfish males in a linear fashion with increasing levels of vitamin C in the diet. Data analysis is not complete, but the highest level diet appears to improve spawning percentage, fecundity, and fry/kg. Overall spawning results were poor and the fish may have been spawned too late in the year. There appears to be a positive impact in the late spawning season, but this study needs to be repeated in the peak spawning season.

Effect of Frozen Forage on Reproduction

In a previous experiment, the benefits of forage were small for increasing the production of hybrid embryos by channel catfish females. In that experiment low numbers of forage were available in the pond, which may explain the lack of strong treatment-related effects. In another evaluation, three strains of channel catfish females were fed twice a week with a commercial diet supplemented with frozen gizzard shad. The common belief is that forage can help prepare channel catfish, which is partially piscivorous, for spawning. Forage did not, however, have a beneficial effect on fry production for any of the three strains (Table 6).

Table 5. Percent of females culled (non-spawnable), ovulated, latency time (hr), fecundity (egg/kg/ovulated female and channel-blue hybrid fry/kg body weight for channel catfish females fed a control diet (37-200 ppm vitamin C), a medium level of vitamin C (500 ppm) or a high level of vitamin C (1,000 ppm).

Diet	Culling %	Ovulation %	Latency time	Fecundity	Fry/kg
Control	41.8	84.4	48.1	4,637	345
Medium	27.3	78.1	43.1	5,681	476
High	17.4	89.5	45.7	7,912	711

Table 6. Channel-blue hybrid fry/kg body weight for 3 strains of channel catfish (one spawned on 2 dates, the second spawning being late in the season) fed a standard commercial diet or the standard commercial diet supplemented with forage, frozen gizzard shad, twice per week.

Treatment	Fry/kg body weight
Strain 1	3,443
Strain 1 forage	2,052
Strain 2 forage	2,489
Strain 2	2,446
Strain 3	1,970
Strain 3 forage	1,484
Strain 3 late	847
Strain 3 late forage	461

Blue Catfish Males and Lipid Source and Ratios (n3:n6)

A study was conducted in 2006 to evaluate the effect on sperm characteristics from fatty acid enrichment of the male blue catfish diet. Rio Grande strain male blue catfish were fed for 6 months either a non-enriched commercial catfish diet or an enriched diet where DHA and arachidonic acid was added. In May 2006, half the males on each diet were given a 50-µg LH-RH implant followed 2 weeks later with a 100-µg implant. Within 3 days after the second implant, brooders from each diet and hormone combination were paired in pens with a channel catfish female and allowed to spawn naturally. Other males were used as sperm donors to fertilize channel catfish eggs obtained by induced spawning and manual stripping. All males were described by weight gain, body proportions (length, head width and girth) and photographed. Gonadosomatic index (GSI), relative percentage of anterior testis, sperm count and motility time were recorded for each sacrificed male.

Diet had no effect on testes development or sperm count, but the motility time of sperm from brooders given the enriched diet was longer. Fertilization rates (% viable eggs at 48-hours post-fertilization) were similar for each diet and implant combination.

Blue Catfish Males and Forage and Soybean-free Diets

In 2007, four diets were compared to determine their impact on blue catfish male reproductive performance. The four diets were a floating catfish feed with soybeans, floating catfish feed without soybeans, the soybean-free feed supplemented with forage fish (chopped frozen gizzard shad), and a forage fish-only feed. The observed mean sperm/kg and sperm/g testes was highest for the forage fish-only feed (Table 7) . Soybean and soybean-free + forage feeds gave the next highest means for these two reproductive traits. The soybean-free diet consistently resulted in the lowest reproductive performance. The poor performance of the soybean-free feed was unexpected based on the hypothesis that removing soybeans would decrease phytoestrogens in the diet, which would result in increased sperm production.

Table 7. Sperm production for blue catfish males fed a standard diet, a diet without soybeans, forage fish only (frozen gizzard shad) or a soybean-free diet supplemented with forage fish.

Diet	Sperm/g of testes ($\times 10^8$)	Sperm/kg body weight ($\times 10^8$)
Control	5.7	5.6
Soybean-free	3.0	3.5
Soybean-free +forage	4.8	5.8
Forage	5.7	6.7

Objective 1c. *Improve hybrid embryo production via genetic enhancement.*

Auburn University

Strain Effects on Hybrid Embryo Production

The channel × blue catfish hybrid grows faster, has more efficient feed conversion, has a higher tolerance for low dissolved oxygen concentrations, and better survival compared to channel catfish. However, economic production of hybrid embryos is problematic. Some strains of channel catfish females or blue catfish males may have reproductive characteristics more suited for production of channel catfish female × blue catfish hybrid catfish embryos than others.

AU-1 channel catfish females produced greater numbers of hybrid fry than AU-7 for 5 consecutive years (Table 8). AU channel catfish lines 1, 3, 5, and 11 consistently produced high numbers of hybrid fry compared AU lines 6-10, 12, and strain 103 over a 3-year period (Tables 9 and 10). AU lines 4 and 13 were only evaluated 2 years, and were high performers one year and low another. Fry/kg for 103 was very low the first 2 years, but improved to average performance levels the third year.

Strain of male blue catfish also affected hatching rate of hybrid embryos and sperm production. Mean sperm/g of testes ranged three-fold among strains one year and was almost 33% different another year. The total mL of sperm/kg body weight and sperm number/kg body weight also varied three-fold among strains of blue catfish. Percent hatch was different for hybrid embryos produced by different strains of blue catfish males. Percent hatch for hybrid embryos fertilized with sperm from, different blue catfish strains were: AU-1, 2.6%; AU-2, 10.4%; AU-3, 10.9%; AU-4, 26.1%, and AU-5, 1.3%. The percent hatch using AU-4 blue catfish males was significantly higher than with the other strains. In another year, AU-1 males had higher hatch than AU-2 males, 15% and 10%, respectively. Genotype-environment interactions were also observed for sperm production. Utilization of genetic variation has the potential to double efficiency and productivity of hybrid embryo production.

The percentage of channel catfish females that were gravid, culled, ovulated and spawned when injected with LHRHa also varied among strains (Table 11). Differences for these four traits were less pronounced when analyzing the data within 3-year-old, 4-year-old and 5-year-old females, with the differences being the lowest among 5-year-old females. Mean latency periods ranged from 40.9 to 48.4 hours and differed among 17 strains of channel catfish females, but on a relative and biological basis, differences are slight, with less than 10 percentage points difference among strains. When the latency data were analyzed by week, and temperature and temperature degree-days are accounted for, these small differences for latency period become insignificant among strains. Mean egg quality scores did not differ among strains of females. However, replication may not have been adequate to show true differences among egg quality scores.

Results at a glance...

- *Using the appropriate genetic line of channel catfish female can double or triple hybrid fry output. Strain of blue catfish male has important effects on sperm production and hatching rate of hybrid embryos.*

Effect of Selection for Body Weight on Hybrid Embryo Production

Six lines of channel catfish females that had been selected for increased body weight for 6 or 7 generations were compared to randomly bred controls for channel catfish female × blue catfish hybrid embryo production. There was no indication of either a negatively correlated response or inbreeding depression for % of females gravid, % culled, ovulation rate, spawning rate, latency period, egg quality, fecundity, and hatching rate. In some cases, there appeared to be a positive correlated response between direct selection for body weight and certain traits. For example, when the select AU-4 line was compared to its randomly bred control line, the select line outperformed the control line for % gravid females (86.1% vs 68.2%), 5 culled females (13.9% vs 31.8%) and % females spawned (75.0% vs 55.7%). With regards to the interrelated traits hatch rate and fry/kg (Table 12), the selected line AU-6 (29.2% and 2,849 fry/kg) showed more than over two-fold increase compared with 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).

Table 8. Fry/kg for AU-1 and AU-4 channel catfish female when injected with LHRHa and hybridized with blue catfish males over a 5-year period.

	Fry/kg female body weight				
Genotype	2001	2002	2003	2004	2005
AU-1	4,598	4,300	1,857	3,105	5,552
AU-7	2,638	2,550	693	1,045	959

Table 9. Percentage of females ovulating, fecundity, and fry/kg for channel catfish female strains when injected with LHRHa and hybridized with blue catfish males in 2003.

Channel catfish female	% ovulation	fecundity (eggs/kg)	fry/kg
AU-1	100	11,047	1,857
AU-2	75	7,133	2,154
AU-3	100	11,997	1,283
AU-4	82	6,545	1,005
AU-5	100	8,790	858
AU-7	73	10,179	693
AU-8	100	9,122	625
AU-9	75	9,438	492
AU-6	90	7,814	395
103	80	7,425	257
AU-10	45	9,575	163

Table 10. Fry/kg for different lines channel catfish female when injected with LHRHa and hybridized with blue catfish males over a 3-year period.

	Fry/kg female body weight		
Line	2002	2003	2004
AU-11	5,570	-	3,421
AU-5	8,500	858	3,136
AU-1	4,300	1,857	3,105
AU-13	3,500	-	3,042
AU-3	4,800	1,283	2,844
103	679	257	2,680
AU-6	-	395	2,570
AU-12	3,550	-	2,566
AU-8	-	625	2,427
AU-4	-	1,005	2,332
AU-9	4,500	492	2,178
AU-10	-	163	1,902
AU-7	2,550	693	1,045

Table 11. Percentage of channel catfish females gravid, culled, ovulated and spawned by strain during 2004 when induced to spawn with 20 and 100 µg/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa). Significant differences existed among strains for % culled and % spawned.

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

In one case, hybrid embryo production exhibited inbreeding or negatively correlated response to selection for increased body. As 3-year-olds (Table 12), AU-1 had the same hatch, but reduced observed fecundity and fry/kg compared to their randomly bred control. As 4-year-olds (Table 13), they had both reduced hatch and fry/kg. In individual years, these differences were not significantly different, but over time, they were different, as the decrease in fry/kg was almost exactly 50% each year. Several possible explanations exist. Selection for body weight decreased reproductive performance and the ability to generate hybrid fry via artificial fertilization techniques and/or reduced the age of first sexual maturity as 3-year-olds. Alternatively, these effects could be attributed to inbreeding depression from mass selection in the select lines that may be accumulating in these relatively small research populations. The results for the 4-year-olds would not likely be due to a reduced age of sexual maturity.

The adverse effects of mass selection for body weight on reproductive performance may be more severe than what these results indicate. Early research at Auburn University indicated that in early generations of individual selection for body weight, positively correlated responses occurred for reproductive traits. In cases where the select lines are not different from controls or have decreased reproductive performance compared to controls, the reduction in performance is even greater if the select lines once had better reproductive performance 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 in our trials. This variable result may have been partially caused by the genetic

Table 12. Mean egg quality, fecundity, hatch %, and fry/kg for selected 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 (C). An asterisk following the entry indicates that there are significant differences in that trait between the select and control lines for that strain.

	Egg Quality		Fecundity (Eggs/kg)		Hatch %		Fry/kg	
Genotype	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-1C	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-7C	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-7C	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-7C	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

Table 13. Ovulation %, mean fecundity (eggs/kg), mean hatch %, and mean fry/kg for AU-1 select and control lines spawned during the 2005 spawning season using a 100 ug implant of luetinizing hormone-releasing hormone analog (LHRHa). Couplet represents a different selected line (top) compared to its randomly bred control (bottom).

	Ovulation %		Fecundity (Eggs/kg)		Hatch %		Fry/kg	
Genotype	N	%	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
AU-1	13	69.2	9	10,581 ± 2,366	9	32.5 ± 31.4	9	3,772 ± 3,443
AU-1 Control	18	61.1	11	10,360 ± 3,776	11	58.2 ± 59.5	11	5,552 ± 4,650

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 lines that are mass-selected 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.

Effect of crossbreeding on hybrid fry output by channel catfish females

The main problem preventing the widespread use of the channel catfish × blue catfish hybrid catfish 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, crossbreeds. In the current experiment, the crossbreed AU-7 × AU-1 was compared to its parental lines to determine if any reproductive benefits were achieved through the use of the crossbreed when forming hybrid catfish. The crossbreed actually exhibited lower ovulation rate (52.1%) than the parental lines (AU-7 = 83.3%; AU-1 = 63.2%), suggesting negative heterosis by the crossbreed for age at maturity (Tables 14 and 15). For 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 rate 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 parental line. There was no advantage to using this crossbred line to form hybrid, since the best overall 2-year performance was exhibited by parental line AU-1.

The lower ovulation and spawning rates obtained from the crossbreed could be the result of negative heterosis for reproductive performance or delayed sexual maturation. This is a surprising result as previous work showed the opposite effect for intraspecific spawning in pens within ponds when ancestors of these lines were compared to their crossbreed. In the previous work, the crossbreed exhibited positive heterosis for early sexual maturation, but there was no difference among genotypes at 4 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 4-year-olds during the 2005 spawning season, and had ovulation % 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 × 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 there was no effect of genotype on latency period when induced to spawn with a 20 μg/kg priming dose of lueitenizing hormone-releasing hormone analog (LHRHa) followed by a resolving dose of 100 μg/kg of LHRHa at 3 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. 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-μg 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 =

Table 14. Percentage of three-year-old females gravid, culled, ovulated, and spawned for AU-1, AU-7, and the crossbreed AU-7 female × AU-1 male channel catfish during the 2004 spawning season when induced to spawn with 20 and 100 μg/kg (priming and resolving dose) luteinizing hormone-releasing hormone analog (LHRHa). Ovulation % and spawning % were significantly different among genotypes. Strains were not different for % culled.

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 × AU-1	47	68.1	31.9	46.9	31.9

Table 15. 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 × AU-1 male channel catfish 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. Ovulation % was different among genotypes for three-year-old and when data from three-year-old and four-year-old females are pooled. Ovulation % was not different for four-year-old females.

	Ovulation%		Egg Quality		Fecundity (Eggs/kg)		Hatch%		Fry/kg	
Genotype	N	%	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Three-Year Old-Females										
AU-1	25	60.0	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	16	3.9 ± 0.8a	16	8,150 ± 4,829a	16	10.8 ± 9.8b	16	801 ± 793b
AU-7× AU-1	22	46.9	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	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			4	12,579 ± 3,196a	4	9.9 ± 15.9a	4	1.592 ± 2.773a
AU-7 × AU-1	16	62.5	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	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	16	3.9 ± 0.8a	20	9,036 ± 3,213a	20	10.7 ± 10.8b	20	959 ± 1,348b
AU-7 × AU-1	48	52.1	18	3.9 ± 0.8a	25	9,282 ± 4,806a	25	16.0 ± 21.0b	25	1,885 ± 2,537ab

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. Apparently, there is either a genotype × age, genotype × delivery method or genotype × temperature interaction for latency period in channel catfish females.

Egg quality scores of the crossbreed AU-7 × 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 3-year-old females had to compete with older females during preparation whereas the 4-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 and 8,200 eggs/kg of female body weight. In 2005, the fecundity of females belonging to all genotypes was higher (range: 10,600 to 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. However, the same researchers found that when using the crossbreeds AR and ARMK, there was an improvement in fecundity over the parental lines. Crossbreeding has variable effects on fecundity as it does for virtually all traits.

Hatching rate and fry/kg were interrelated. During the 2004 spawning season, there was a significant effect of genotype on the hatching rates of eggs with the parental strain AU-1 having significantly higher hatches than either the parental line AU-7 or the crossbreed. Hatch rate 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 3 years old, but very similar to the crossbreed at 4 years of age.

When the data from 3-year-old and 4-year-old females was combined, there was also an effect of genotype on hatch. The parental line AU-1 had a hatch rate between two and three times that seen by either the parental line AU-7 or the crossbreed AU-7 × AU-1.

Results were essentially parallel for fry/kg. Three-year-old females belonging to the genotypes AU-7 (801 fry/kg) and AU-7 × AU-1 (880 fry/kg) produced significantly fewer fry/kg of female body weight than genotype AU-1 (2,135 fry/kg). No statistical significant differences in fry production were observed during the 2005 spawning season once all females had achieved 4 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 hatching rates, the observed result was that AU-1 had two to three times the fry production of AU-7 both years, triple that of the crossbreed when 3 years old, but very similar to the crossbreed at 4 years of age. Also, similar to hatch rate, when the data is pooled for 3-year-old and 4-year-old females, AU-1 produces approximately three times as much fry/kg than AU-7, and about two times as many as AU-7 × AU-1.

One explanation for the difference in hatch rates 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 rates and fry production values during the 2004 spawning season. However, the maternal effect may only be evident at younger ages, since once females achieved 4 years of age, all genotypes examined have statistically identical hatching rates and fry production.

Reproductive traits often exhibit heterosis and can be improved with crossbreeding; however, there was no heterosis observed in this experiment. At 3 years of age, there was the possibility of dominance from the poorer performing AU-7 parent, but this genetic mechanism was not evident at 4 years of age.

The parental lines were potentially inbred and may have been exhibiting inbreeding depression for reproductive performance. The data from the 3-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 broodfish 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 as dams. The best 2-year performance would be obtained from using the AU-1 line as dams.

Effect of crossbreeding on sperm production of blue catfish males

During 2006, the early sexual maturity of 4-year-old blue catfish male crossbreeds (AU-1 × AU-2) was examined. These fish exhibited no signs of early sexual maturity, and had extremely small testes.

These same fish were evaluated again in 2007. Additionally, reciprocal AU-1 × AU-2 crossbreed males were compared to AU-1 males, the parent strain with the greatest sperm output. There was no heterosis exhibited by either crossbreed for sperm/kg body weight or sperm/g testes, thus there are no apparent advantages for using crossbred blue catfish males to make hybrid embryos Table 16.

Mississippi State University. Groups of nine, 2-year-old female channel catfish brood stock obtained from each of four different strains/sources were tagged and stocked into four, 0.04-ha earthen ponds (36 fish per pond, 9 fish per strain) in April. Blood and egg samples were collected from twelve fish in each pond (3 fish/strain) every month for 11 and 9 months for blood and eggs, respectively. No individual fish within a strain was subject to sampling more than once every four months. Plasma estradiol, plasma testosterone, cathepsins, protein content of eggs and egg size were measured. No noteworthy differences in the mean values of the physiological indices monitored were observed among the four strains during each month.

Table 16. Sperm production for AU-1 blue catfish males and its reciprocal crossbreeds with AU-2 blue catfish (female × male).

	Sperm/g of testes ($\times 10^8$)	Sperm/kg body weight ($\times 10^8$)
AU-1	5.0	6.5
AU-1 × AU-2	5.1	5.1
AU-2 × AU-1	4.0	3.8

Objective 2. *Develop induced spawning techniques and management strategies to optimize gamete collection and storage.*

Objective 2a. *Develop procedures to predict ovulation of channel catfish.*

Auburn University. Hybrid channel × blue catfish can be obtained by induced spawning and artificial fertilization but with variable results. A threshold degree of maturity must be reached before broodfish can be induced to spawn, but selection of such fish can be very subjective. Temperature of the surrounding environment affects the rates of physiological processes in fish. Response time to

applications of induced spawning hormones such as LHRHa is thought to be related to water temperature.

Female broodfish (Marion strain channel catfish) were given a subjective ranking of poor, fair or good as well as measurements of body weight, total body length, body width and girth were taken. Broodfish were held at 24, 26, and 28°C in 60-gallon aquaria and injected with LHRHa at 20 μg/kg as a preparatory injection followed 12 hours later with 100 μg/kg. Fish were monitored hourly as ovulation approached, and the time of the first egg deposit and when approximately 100 eggs were found were recorded. Approximately half the females were manually stripped soon after the first egg was observed, and the other fish were stripped 4 to 6 hours after the first egg was observed. Eggs were artificially fertilized with blue catfish sperm and incubated. For each egg mass, the percentage of viable embryos at 24 hours after fertilization, the percent hatch, and percent survival at swim-up was determined.

The overall mean degree-hour response time (temperature in degrees Celcius multiplied by the time in hours to first egg release) was 1,156 ± 275. The mean degree hour response time was 1,416 ± 107 at 24°C, 1,228 ± 211 at 26°C and 981 ± 278 at 28°C. The percentage of females that ovulated were 58, 62.5 and 87.5% at 24, 26, and 28°C, respectively. The majority of females which did ovulate did so between 58 to 64 hours at 24°C, 48 to 52 hours at 26°C and 24 to 40 hours at 28°C with the fish classified as "good" spawning sooner than the "poor" classification at all temperatures. When only the good quality females were considered, the weight of eggs released/kg female varied by water temperature. At 24°C an average of 70 ± 60 g were obtained/kg, at 26°C 126 ± 41, and at 28°C 154 ± 34. The number of eggs/g of eggs also varied by temperature: 71 ± 11, 53 ± 6, and 48 ± 10 at 24, 26, and 28°C respectively. Egg quality varied with how soon eggs were taken after the first egg was released. For females at 28°C, when eggs were taken within 2 hours of being observed the % viable embryos averaged 76 ± 13% and the hatching rate was 31 ± 16%. When eggs were taken at 4 or more hours of being observed, the % viable embryos averaged 66 ± 19% and the hatching rate was 9.7 ± 6.6%. When a female was stripped within 2 hours after the first eggs were released, a lower weight and total number of eggs/kg (107.3 ± 46.6 and 5,739.8 ± 2174) were obtained relative to fish stripped 4 or more hours after the first eggs were released (147.7 ± 36 and 7,724 ± 2,120, respectively).

Results at a glance...

- *Hatching rate of hybrid embryos is improved if LHRH-injected channel catfish females are stripped within 2 hours of first observation of egg release. Waiting longer will increase the number of eggs stripped, but this is more than offset by much lower hatch rate.*

Proper selection of broodfish for induced spawning can help insure a high rate of spawning success and good egg quality. However, the brood selection is often subjective based on general appearance of the fish and the culturist's experience. Appropriate quantitative criteria can reduce individual bias and assists the less experience biologist in brood selection. Five trials were conducted using 3- and 5-year-old channel catfish females where the physical characteristics of total length, weight, and width were measured and ratios calculated. Development and pulsation of the genital papilla was also used as a point of evaluation. Females were induced spawned using LHRHa at 120 μg/kg and manually spawned. Eggs were artificially fertilized with blue catfish sperm. Spawning success, and egg production characteristics were evaluated as to their

relationship to brood stock characteristics.

Age of broodfish had a significant effect on spawning success. Of the 5-year-old fish, 91% spawned while only 26% of the 3-year-old fish spawned. The two age groups also differed in average weight which was a factor influencing spawning success. Fish weighing over 3 kg had an average spawning rate of 80% while fish weighing 3 kg or less averaged 20%. Fish that were 60 cm, or more, in total length, had a spawning rate of 80%. Fish with a length (cm)/weight (kg) ratio less than 15 also averaged an 80% spawning rate. The length (cm)/width (cm) ratio did not exhibit a well-defined pattern, however, fish with a ratio less than 5 had a 60% success rate. Fish with a width (cm)/weight (kg) ratio less than 4 have 75% chance of spawning. Whether or not the genital papilla was pulsating at the time of the first LHRHa injection had no relationship to spawning success. Brood age affected egg characteristics. Younger fish had more eggs per gram of eggs. The mean number of eggs/kg for 3-year-old fish was 8274 ± 2868, while the mean for 5-year-old females was 4842 ± 1130. The 3-year-old fish ovulated, on average, 9.7 hours later than 5-year-old fish. In general, variations in other brood stock descriptors were not associated with variations in eggs per gram, number of eggs per kilogram body weight, time of ovulation, or viability. However, egg diameter was related to length (cm)/ weight (kg) ratio with larger fish producing larger egg diameters than smaller fish.

Results at a glance...

- *Brood stock age is important for spawning success. Other indicators of spawning success are broodfish weight, length, and ratios of body proportions. Broodfish having a width (cm)/weight (kg) ratio of less than 4 have a high spawning success rate. To obtain the best success in induced spawning to produce hybrids, broodfish should be 5 years old, with a weight of over 3 kg (6 to 7 pounds), and a length greater than 60 cm (24 inches).*

In this study, brood stock age was the most important consideration for spawning success. Related to age were brood weight and length and their effect on spawning success. Ratios of body proportions also were related to spawning success with fish having a width (cm)/weight (kg) ratio of less than 4 having a greater than 70% success rate. To obtain the best induced spawning success broodfish should be selected to be 5 years old, with a weight of over 3 kg, and a length greater than 60 cm. Such fish should give a 94% spawning rate.

Objective 2b. *Conduct pivotal protocol studies for determining dosage rates and timing of application of luteinizing hormone releasing hormone (LHRHa), carp pituitary extract and catfish pituitary extract to maximize ovulation, hatch rate and fry production.*

USDA-ARS. The effectiveness of catfish pituitary extract, carp pituitary extract, and LHRHa for inducing spawning in female channel catfish and subsequent production of channel catfish × blue catfish hybrid fry was compared. Mature female catfish (3 to 5 years old) were injected with carp pituitary extract (N = 66), catfish pituitary extract (N = 51), or LHRHa (N = 58). Catfish pituitaries were collected in March and April at a commercial catfish processing plant from fish larger than 3 pounds, dried in acetone, and ground to a powder. Carp pituitary and LHRHa were purchased from commercial vendors (Stoller Fisheries, Spirit Lake, Iowa and Syndel International, Inc., Vancouver, British Columbia, Canada, respectively). Injection regimes were 2 mg/kg female body weight (BW)

initial injection and 8 mg/kg 20 hours later for carp and catfish pituitary extract or 40 μg/kg female BW initial injection followed by 80 μg/kg 20 hours later for LHRHa. Females were checked for ovulation 24 hours following the final injection. Ovulating females were tranquilized and eggs were manually stripped into Hank's Balanced Salt Solution (HBSS). Eggs were weighed and then fertilized with blue catfish sperm. Blue catfish sperm was prepared by macerating testes from 4 to 5 blue catfish males and pooling the sperm in HBSS. Approximately 25 mL of sperm-solution was used to fertilize each 400-g sample of eggs. Egg masses were placed in hatching troughs following fertilization and percent viable embryos was determined at 48 hours post-fertilization. Fry numbers at hatch were estimated volumetrically. Data collected for each treatment included: weight of females injected, percent of injected females that ovulated, fecundity (number of eggs/kg female body weight), percent viable embryos at 48 hours, fry/kg body weight of all females, fry/kg body weight of ovulated females, and total fry.

There were no differences among treatments for any of the variables measured (Table 17). Results demonstrate that catfish pituitary extract was as effective as carp pituitary extract or LHRHa for inducing ovulation in channel catfish females. Catfish pituitary is readily available from commercial catfish processing facilities, although regulatory issues associated with using it to induce spawning in fish are not known.

Eggs from females ovulated with LHRHa flowed much easier and more completely, but it seemed their time-frame for ovulation was wider. The LHRHa may have done better if a longer period of time would have been allowed for ovulation. The pituitary-treated fish seemed to ovulate more synchronously, but never flowed as well as a good LHRHa fish. This observation that CPE-treated fish ovulate more synchronously has been confirmed at Auburn University. Latency time for LHRH-treated fish is longer, and the observations observed at USDA are consistent with observations at other locations.

Auburn University. The 2004 research was conducted with Good Manufacturing Practices (GMP) grade LHRHa for injections and research grade LHRHa for implants at Auburn University. The dose 30/150 (μg/kg priming/resolving dose) was the most effective injection treatment confirming earlier results with research grade LHRHa. (All doses are reported as total micrograms of product injected. The peptide content of the product is 82% LHRHa. Thus, a 100 μg dose of ingredient is actually 82 μg of

Table 17. Comparison of catfish pituitary extract, carp pituitary extract, and LHRHa for inducing spawning in channel catfish females and production of channel catfish × blue catfish fry.

Treatment	# of females injected	Mean weight of females (kg)	% females ovulating	Eggs/kg female BW	% viable embryos	Fry/kg BW all females	Fry/kg BE ovulated females	Total fry
Carp PE	66	2.9	71.0	6,482	55.5	1,348	1,788	239,100
Catfish PE	51	2.8	68.0	6,767	64.1	1,128	1,600	190,100
LHRHa	58	3.0	65.0	6,482	66.3	1,527	1,999	254,100
Standard Error		0.2	8.4	720	8.6	344	350	

LHRHa.). This peptide content is the same for all experiments for all institutions conducting research in this SRAC project. The efficacy of the 30/150 injection and the 100-µg implant were not different. Females that were not hormone induced and held in ponds or for short periods in tanks did not ovulate. All LHRHa treatments were effective yielding a minimum of 64.7% ovulation.

The highest observed means for ovulation percentage were the 125-µg implant and the 20/100 injection (Table 18). However, the treatments yielding the most fry/kg female body weight were the 100-µg implant and the 30/150 injection.

Early in the spawning season implants gave more consistent results than injections, but the best injection regime (30/150) was not different from the best implant regime (100 µg) (Table 19). Ovulation of females in individual units was more effective than in a communal group, and in absence of conspecific males was more effective than in the presence of conspecific males. In terms of gamete release, all treatments were highly effective with ovulation % ranging from 66.6 to 100.0% with a grand mean of 86.1%. However, gamete quality differed among treatments as indicated by the variation in fry/kg (Table 19).

During the peak spawning season, the ovulation rates decreased (Tables 20 and 21). In terms of gamete release, most treatments were effective with ovulation % ranging from 28.6 to 71.4% with a grand mean of 54.9%. However, gamete quality differed among treatments as indicated by the variation in fry/kg. Again, 30/150 injection and the 100-µg implant were the most effective treatments and not different from each other. Fry output, approximately 1700 fry/kg, of these two better treatments was similar to the output in the early spawning period. Results were similar for the early and peak spawning (Tables 19, 20, and 21).

Table 18. Ovulation percentage and hybrid fry/kg female body weight for channel catfish and females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. Dose 0 is primarily from females held in ponds and some in tanks.

LHRH Dose	N	Ovulation %	Fry/kg
0	555	0.0	-
10/50	24	75.0	500
20/100	500	76.3	1,260
30/150	24	66.6	1,750
75 implant	32	68.9	580
100 implant	34	64.7	1,728
125 implant	10	100.0	955

Table 19. Early season ovulation percentage and hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. The hatching environment was extreme as fungal infection was extremely heavy. Low-producing line of channel catfish was utilized.

LHRH Dose	Environment	N	Ovulation %	Fry/kg
10/50	tank	10	80	0
20/100	tank	10	100	0
20/100	bag	9	78	2,293
30/150	tank	10	90	1,727
75 implant	tank	10	80	492
100 implant	aquaria w/male	7	71	1,650
100 implant	aquaria no male	6	67	1,831
125 implant	tank	10	100	955

Table 20. Peak season ovulation percentage and hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. Low-producing line of channel catfish was utilized.

LHRH Dose	Environment	N	Ovulation %	Fry/kg
10/50	bag	7	71.4	800
20/100	tank	7	28.6	502
20/100	bag	7	71.4	855
30/150	bag	7	57.1	1,736
75 implant	tank	7	42.8	858
100 implant	bag	7	71.4	1,704
75 implant	bag	7	42.8	536

Table 21. Comparison of early and peak season hybrid fry/kg female body weight for channel catfish females fertilized with blue catfish sperm. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research grade LHRHa implants (µg/kg) were used. Low-producing line of channel catfish was utilized.

	Fry/kg	
LHRH Dose	Early	Peak
10/50	0	800
20/100	0	855
30/150	1,740	1,736
75 implant	492	858
100 implant	2,000	1,704

Results at a glance...

- *The dose of 100 µg/kg LHRHa implants results in the most consistent hybrid fry production. At the end of the spawning season this dose needs to be reduced to 75 µg/kg.*

In two additional studies during the peak spawning season, 100-µg implants resulted in about triple the number of fry/kg compared to other treatments (Tables 22 and 23). Additionally, a single injection of 150 µg/kg of liquid LHRHa was no more effective than 50-µg implants.

During the late spawning period, the injection regime

Table 22. Mean percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females by experiment after injection (priming dose (µg/kg)/resolving dose)or implantation (single implant, µg/kg) with luteinizing hormone releasing hormone agonist, LHRHa when hybridized with blue catfish males. Water temperature averaged 27.5ºC. Means in a column followed by different letters are significantly different (P<0.05).

Treatment	Females	Ovulation %	Latency (hrs)	Fry/kg	Egg Masses	Egg Quality	% Hatch
10/50	7	71.4	44.6ab	862b	21	2.5c	29.9
20/100	7	71.4	40.9b	1,341b	26	3.4b	49.7
30/150	7	43.0	43.5ab	1,120b	16	3.9ab	44.1
75 implant	5	40.0	45.2a	634b	10	3.7ab	28.0
100 implant	5	80.0	42.8ab	3,394a	15	3.9ab	39.4

Table 23. Mean percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females by experiment after injection (priming dose/resolving dose, µg/kg) or implantation (single implant, µg/kg) with luteinizing hormone releasing hormone agonist, LHRHa when hybridized with blue catfish males (mean ± SD). Water temperature averaged 24.9 ºC.

Treatment	Females	Delivery Method	Mean Percent Ovulation	Latency Time	Fecundity	Fry per Kilogram	Egg Masses	Egg Quality	Percent Hatch
50	10	Implant	100	83.2a	12,452	1,500b	34	3.6	19.1a
100	10	Implant	90	78.0ab	12,198	3,846a	34	3.7	34.3a
150	10	Injection	60	69.2b	10,178	831b	22	3.4	19.0a

with the highest ovulation rate was 10/50 at 100% using high-line females (Table 24). The implant with the highest ovulation rate was 75 μg at 85.7%, again for the high-line females. No hatch was obtained in the last spawning period probably because of poor quality sperm or an error in sperm preparation. Genetics had an impact on ovulation. High-line females had higher ovulation than low line females. However, egg quality data was obtained indicating genetics and implants had positive effects on egg quality and presumably hatch (Tables 25-29).

A second experiment was conducted during the late spawning period comparing 75-μg implants with 10/50 injections. Ovulation rates were not different, 91.4% and 91.6%, for implants and injections, respectively. Hatch, 29%, was much higher for 75-μg implant than for 10/50 injection, 12% (Table 30). In a third run, 28 out of 32 individual fish (87.5%) implanted with 75-μg ovulated, with 75.6% hatch.

Table 24. Late season ovulation percentage for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags within tanks without channel catfish males. GMP-grade LHRHa injections (priming dose/resolving dose, μg/kg) or research-grade LHRHa implants (μg/kg) were used. Low- or high-producing lines of channel catfish were utilized.

LHRH Dose	Line	N	Ovulation %
100 implant	high	7.0	71.4
75 implant	high	7.0	85.7
30/150	low	7.0	28.6
20/100	low	8.0	25.0
10/150	low	8.0	25.0
100 implant	low	7.0	42.8
75 implant	low	8.0	25.0
10/50	high	4.0	100.0
10/50	low	3.0	33.3

Table 25. Egg quality and hybrid fry/kg female body weight during the early season for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks. GMP grade LHRHa injections (priming dose/resolving dose, μg/kg) or research-grade LHRHa implants (μg/kg) were used. Low-producing line of channel catfish was utilized. R = 0.23 ($P > 0.05$) between egg quality and fry/kg.

HRH Dose	N	Egg Score	Fry/kg
10/50	10	3.7	0
20/100	10	4.3	0
30/150	10	4.3	1,727
75 implant	10	4.2	492
100 implant	13	4.0	1,831
125 implant	10	3.6	955

Table 26. Egg quality and hybrid fry/kg female body weight during the peak season for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags. GMP grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used. Low- producing line of channel catfish was utilized. R=0.67 ($P < 0.05$) between egg quality and fry/kg.

LHRH Dose	N	Egg Score	Fry/kg
10/50	7	2.5	800
20/100	7	3.3	855
30/150	7	3.9	1,736
100 implant	7	3.9	1,704
75 implant	7	4.1	850

Table 27. Egg quality during the late season for channel catfish females fertilized with blue catfish sperm. Females were ovulated individually in bags. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used.

Dose	Line	N	Egg Score
20/100	low	4	3.0
30/150	low	5	3.4
75 implant	low	5	3.8
75 implant	high	18	4.1
100 implant	low	12	3.2
100 implant	high	20	3.6

Table 28. Percentage of eggs good or bloody during the early (E), peak (P) and late (L) season for channel catfish females fertilized with blue catfish sperm. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used.

Dose	Line	% Good			% Bloody		
		E	P	L	E	P	L
10/50	low	30	16	-	63	63	-
10/50	high	-	-	0	-	-	0
20/100	low	40	13	25	40	81	75
30/150	low	37	33	20	57	58	100
75 implant	low	46	44	60	58	56	80
75 implant	high	-	-	40	-	-	55
100 implant	low	39	29	42	47	37	75
100 implant	high	-	-	40	-	-	35
125 implant	low	32	-	-	50	-	-

Table 29. Percentage of eggs either "good" or "bloody" during the early (E), peak (P) and late (L) season for channel catfish females fertilized with blue catfish sperm. GMP-grade LHRHa injections (priming dose/resolving dose, µg/kg) or research-grade LHRHa implants (µg/kg) were used.

Dose	Line	% White			% Clumps		
		E	P	L	E	P	L
10/50	low	0	47	-	10	89	-
10/50	high	-	-	0	-	-	0
20/100	low	0	0	50	0	72	75
30/150	low	0	0	40	8	42	20
75 implant	low	4	0	0	4	28	40
75 implant	high	-	-	10	-	-	15
100 implant	low	0	0	25	26	50	5
100 implant	high	-	-	0	-	-	25
125 implant	low	0	-	-	35	-	-

Table 30. Effect of LHRH implants on the late season ovulation rate and hatch rate for hybrid catfish embryos. Means differed significantly.

Dose	N	Delivery	Ovulation (%)	Hatch (%)
75	59	implant	91.4	29.0a
10/50	25	injection	91.6	12.0b

Egg quality data was measured subjectively on a 5-point scale (Tables 25-29). Traits included overall quality or % good, bloodiness, whiteness and clumpiness, the latter three being negative traits. Early in the season there was little difference in egg quality obtained from fish with different dosages of injections or implants. There was a relationship between subjective egg quality and hatch. Early in the season the correlation between egg quality and hatch was 0.23. By the peak spawning period differences in egg quality emerged. Egg quality was higher for implants than injections. The correlation between egg quality and hatch increased to 0.67.

In the late spawning season differences in egg quality still existed. Again implants tended to have higher mean values than injections. Genetics impacted the results. High-line females had higher observed means than low lines.

Implanted fish had a more variable time of ovulation, but females that ovulated up to 48 hours later than the average female gave high quality eggs, whereas late ovulating injected females give over ripened eggs. The advantage of the implants is greatest late in the spawning season.

Latency period after initial injection was longer in the early season at lower temperatures (Table 31). Higher doses tended to give shorter and more uniform latency periods. Injections produced shorter latency. In general, the higher doses yielded a higher number of fry per kilogram of female until late in the season when the lower doses provided maximum effectiveness. The LHRHa implants were more effective than the injections at producing channel catfish female × blue catfish male hybrid embryos. Two treatments, 100 μg/kg implants and 30/150 μg/kg injection, yield the greatest number of fry/kg. Of those two, 100 μg/kg implants was the most consistent treatment, and had the maximum mean fry/kg. Late in the spawning season 75 μg/kg implanted females had both higher ovulation rate

Table 31. Mean latency period (hours ± standard deviation, SD) for female body weight for channel catfish females fertilized with blue catfish sperm. Females were ovulated communally in tanks, individually in bags within tanks or individually in aquaria with or without channel catfish males. GMP-grade LHRHa injections (priming dose/resolving dose, μg/kg) or research grade-LHRHa implants (μg/kg) were used. No resolving dose treatments are implants. Batch = nutrition or injection (dose) experiments.

Date	Batch	Priming	Resolving	N	Latency	SD
5/20	Injection	20	100	3	45.72	5.56
5/20	Injection	30	150	1	43.80	
5/21	Injection	10	50	8	55.81	3.35
5/21	Injection	20	100	14	51.61	1.01
5/21	Injection	30	150	8	51.17	0.31
5/21	Injection	75	0	8	57.45	0.5
5/21	Injection	100	0	9	56.42	3.77
5/21	Injection	125	0	10	56.51	0.98
5/26	Nutrition	20	100	75	43.65	1.58
5/27	Nutrition	20	100	69	40.80	1.74
6/3	Nutrition	20	100	55	50.27	1.98
6/11	Injection	10	50	5	44.06	2.14
6/11	Injection	20	100	7	40.89	4.36
6/11	Injection	30	150	3	40.43	3.52
6/11	Injection	75	0	5	43.50	3.86
6/11	Injection	100	0	4	41.75	2.2
6/16	Nutrition	20	100	64	42.35	3.17
6/17	Nutrition	20	100	57	39.85	2.1
6/18	Nutrition	20	100	41	38.88	2.72
6/23	Injection	10	150	2	40.35	4.17
6/23	Injection	20	100	2	39.35	0.07
6/23	Injection	30	150	2	43.55	2.76
6/23	Injection	75	0	8	39.8	2.81
6/23	Injection	100	0	8	38.09	1.36
6/24	Injection	10	50	5	50.28	2.42

and egg quality compared to females implanted with 100 μg/kg of LHRHa. Latency time decreased with increasing temperatures.

A study was conducted in the 2006 season to evaluate the value of LHRHa implants for blue catfish male reproductive characteristics. Males were fed either a control diet or a fatty acid enriched diet. In May 2006, males were given a 50-μg LH-RH implant followed two weeks later with a 100-μg implant. All males were described as to their weight gain, body proportions (length, head width and girth) and photographed. GSI, relative percentage of anterior testis, sperm count and motility time were recorded for each sacrificed male.

The use of LHRHa implants had no effect on testicular development but sperm count/mL was greater from implanted males as was motility time. Fertilization rates (% viable eggs at 48-hours post-fertilization) were similar for each diet and implant combination.

In a second experiment, different strains of blue catfish males were given a 100-μg/kg implant and then sacrificed after 48 hours. Results were not consistent across strains. Some strains had increased sperm production, but most did not. Although not statistically significant, in 4 of 5 strains there was a trend of increased hatch rate for embryos from implanted males.

University of Memphis. Channel catfish ovarian follicles were treated in vitro with 17α, 20β-dihydroxyprogesterone (DHP) and human chorionic gonadotropin in vitro. Initial efforts have focused on screening for potentially effective hormones to influence oocyte maturation and ovulation. Evaluations have included various culture media, hormonal concentrations, and the timing of the application of hormones. Methods are being investigated to adequately evaluate the oocyte response to various treatments. Such findings will hopefully be applicable to the evaluation of gonadotropins used to induce spawning of eggs of high quality from channel catfish brood stock.

Trials were conducted of in vitro oocyte maturation in early June 2006 on several ovaries. The largest size classes of oocytes were dissected from the ovaries as ovarian follicles (i.e. with surrounding follicle wall) and placed into Cortland's balanced fish saline. Groups of 5 follicles were placed into wells of a 24-well plate containing 2 mL of Cortland's per well. Steroids were administered in ethanol:propylene glycol (1:1). Trials in previous seasons used an incubation time up to 24 hours; this year we extended the incubation for an additional day and found indications of successful oocyte maturation (Figure 2). There was a dose-related increase in translucency over time with most of the ooplasmic clearing occurring in the second day of incubation.

While the greatest change in translucency occurred at the highest doses of DHP, the vehicle alone also showed increased translucency indicating a high basal level of spontaneous maturation (Figure 2). Animation of image stacks revealed the quiescent nature of oocytes during maturation (no equivalent cytoplasmic movements that we have seen in activated eggs and embryos). Nevertheless, some favorably positioned oocytes that underwent clearing did show what appeared to be blastodisc formation (ooplasmic streaming to one pole).

Auburn University. Reducing handling and stress of channel catfish females may be key factors for effective production of channel catfish female × blue catfish hybrid catfish embryos. Females were either left free in tanks or confined in bags or aquaria. Confinement increased hybrid fry production and reduced labor involved in the production protocol. Exposure to the scent of conspecific males increased, decreased or did not affect hybrid fry production (Tables 32, 33, and 34). Method of exposure appears to have an effect. Positive effects on hybrid fry production were obtained when water from tanks containing males is

introduced, whereas visual or actual contact appears to have negative effects (Tables 33 and 34).

These experiments were repeated. Direct exposure of females to the scent of conspecific males appeared to have a positive effect on fry production (Tables 35 and 36). Indirect exposure had a stronger and positive effect on fry production. Curiously, females that were directly exposed to males had an increased latency of about 6 hours.

Figure 2. Effect of 17α, 20β dihydroxyprogesterone (DHP) on in vitro channel catfish oocyte maturation as determined by computer-aided meiotic maturation assay (CAMMA). Dose of DHP in µg/ml. Temperature on scanner bed 28°C. Each treatment represents 20 follicles (4 replicate wells with 5 follicles each).

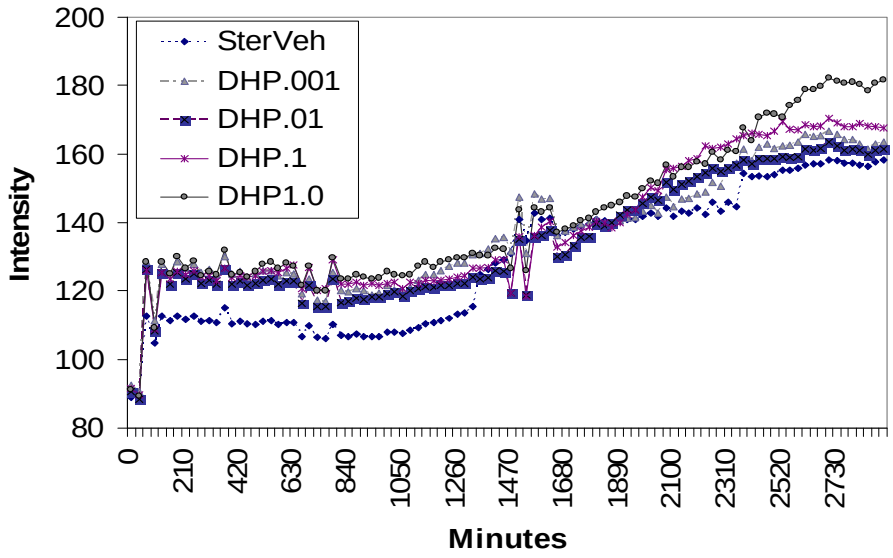


Table 32. Mean eggs/kg female body weight (BW), hatching percentage, fry/kg female body weight and egg quality of channel catfish females exposed or not exposed to channel catfish male after injection with luteinizing hormone releasing hormone, LHRHa, when hybridized with blue catfish male (mean ± SD) in 2001. Means followed by the same letter are not different (*P*>0.05) within each column.

Treatment	Spawning Percentage (N=24)	Egg/kg Female BW	Hatching Percentage	Fry/kg Female BW	Latency Time (hour)	Egg Quality
Unexposed	90a ± 30	6,822a ± 2,268	31.1a ± 6.7	2,246a ± 652	31a ± 5	3.3a ± 0.2
Exposed	100a ± 0	7,358a ± 1,756	40.5b ± 1.6	3,031b ± 1,028	30a ± 5	3.7b ± 0.1

Table 33. Mean spawning percentage, egg/kg female body weight (BW), hatching percentage, fry/kg female body weight and latency time at 29°C for channel catfish females injected with luteinizing hormone releasing hormone, LHRHa, with different exposures to channel catfish males (mean ± SD) in 2002. Means followed by the same letter are not different (*P*>0.05) within each column.

Treatment	Spawning Percentage (N =10)	Egg/kg Female BW (N =10)	Hatching Percentage (N = 10)	Fry/kg Female BW (N = 10)	Latency Time (hour) (N = 10)
30 + 150 low male	80a ± 42	9,368a ± 1,519	14.4a ± 0.64	1,351a ± 219	31a ± 0.10
30 + 150 no male	80a ± 42	8,288a ± 2,671	52.9b ± 0.45	4,384b ± 1413	31a ± 0.10
30 + 150 high male	90a ± 31	8,211a ± 3,882	23.2c ± 0.11	1,901a ± 899	32b ± 0.52

Table 34. Ovulation % and fry/kg female body weight for channel catfish receiving 100-µg LHRHa implants either in direct contact with or not exposed to conspecific males and fertilized with blue catfish sperm.

LHRH Dose (µg/kg)	Environment	N	ovulation %	Fry/kg
100	aquaria w/male	7	71.4	1,650
100	aquaria no male	6	66.6	1,831
100	bag no male	7	71.4	1,704

Table 35. Percent ovulation, mean latency period, and fry per kilogram of female for channel catfish females receiving 100-mg LHRHa implants when hybridized with blue catfish males males (mean ±SD). Females were not exposed to males (N), directly exposed to males (YD), or indirectly exposed (YI) to males. Means followed by the same letter are not significantly different (*P*>0.05).

Year	Male Exposure	N (Females)	Mean Percent Ovulation	Latency	Fry per Kilogram of Female
2004	N	170	62.4	47.2b± 6.8	534 ± 1289
	YD	8	62.5	53.1a± 2.7	1449 ± 0
2005	N	165	62.2	62.6a± 14.5	1,350 ± 1,660
	YI	10	50.0	62.8a ± 9.7	4,322 ± 853
	YD	32	78.1	69.0a± 10.5	2,188 ± 2,440

Table 36. Percent ovulation, mean latency period, fecundity, fry per kilogram of females, and percent hatch for female channel catfish receiving 100-mg LHRHa implants when hybridized with blue catfish males (mean ± SD). Females were not exposed (N), directly exposed (YD) or indirectly exposed (YI) to males in aquaria. Means followed the sames letter are not significantly different (*P*>0.05).

Treatment	N (Female)	Delivery Method	Mean Percent Ovulation	Latency	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
N	5	Implant	80	67.3	13,793	5,321	22	NA	46.1
YD	32	Implant	78.1	69.0	10,676	3,232	101	4.3	41.5
YI	10	Implant	50	62.8	11,944	6,649	25	3.8	62.0

Objective 2d. *Develop extended refrigerated storage and cryopreservation of sperm.*

Louisiana State University. Knowledge of sperm concentration is essential for standardization of protocol for gamete cryopreservation and for optimizing fertilization in artificial spawning. Currently there is a lack of information regarding sperm concentration and how it relates to cryopreservation and fertilization in essentially all species including channel catfish. Practical methods for evaluation of sperm concentration in channel catfish are needed. The specific objectives of this study were to evaluate: 1) the use of a spectrophotometry in determining sperm concentrations; 2) sperm concentrations relative to gonad composition, and 3) optimal sperm concentration for fertilization during artificial spawning.

Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hanks' balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1 g/20 mL) to release sperm.

Sperm concentrations and motility estimates relative to gonad composition are summarized in Table 37. Sperm concentrations varied in relation to gonad composition.

Table 37. Summary of sperm concentrations and motility from whole testis and posterior and anterior sections. Means in a column with different letters were significantly different (*P*<0.05, n=21).

	Concentration (/mL)	Total Concentration	Sperm/g Testis	Motility (%)
Intact	$1.73 \times 10^8 \pm 9.4 \times 10^7$a	$1.78 \times 10^{10} \pm 2.0 \times 10^{10}$a	$3.52 \times 10^9 \pm 1.89 \times 10^9$a	35±4.5a
Posterior	$1.06 \times 10^7 \pm 2.7 \times 10^7$b	$1.41 \times 10^8 \pm 2.37 \times 10^8$b	$2.09 \times 10^8 \pm 5.4 \times 10^8$b	23±4.6a,b
Anterior	$3.13 \times 10^8 \pm 1.18 \times 10^8$c	$1.42 \times 10^{10} \pm 1.5 \times 10^{10}$c	$5.74 \times 10^9 \pm 2.24 \times 10^9$c	41±4.6b

Auburn University. Research on the production of channel catfish × blue catfish hybrid embryos and on other genetic improvement programs would be enhanced by more effective refrigerated storage of sperm. Bacterial growth is one of the main causes of death for sperm during refrigeration. Using antibiotics to improve sperm condition while refrigerated could increase sperm concentrations and ultimately the number of fry produced or would allow for more complicated mating designs. Blue catfish sperm were stored 4 days with and without gentamyacin. When antibiotics were applied to the sperm the percent hatch increased 2.5 times. In another experiment sperm refrigerated with gentamyacin gave high hatch rates after 21 days of storage. Antibiotics could allow refrigeration to become a way to store viable sperm for several days without the sperm dying from bacterial infections.

Objective 2e. *Develop short-term extended storage of eggs*

University of Memphis. In our continuing study of egg quality, activation and fertilization, the effects of various osmoticants ("extenders") were tested on channel catfish eggs obtained from hormone-induced females at the USDA Stoneville, Mississippi facility, Spring 2007. The major osmoticant tested was Hank's balanced salts solution at various concentrations ranging from 75 to 125%. In addition, simple 0.15M NaCl saline, a fish Ringers (Cortland's solution) and various sucrose concentrations along with protein additives including bovine serum albumin and ovalbumin were tested. Analysis of the results is currently ongoing.

To determine the effect of saline solutions on catfish eggs, unfertilized eggs were treated with different salt solutions, some in combination with other osmoticants (e.g., protein and sucrose) and the eggs were imaged by transparency scanners. All groups showed chorion elevation or expansion and increase in the perivitelline space, even in the Hank's salts treated groups (Figure 3). Since egg diameters rapidly increased in the water-added groups, compared to the others tested, additional measurements were made at 2 minute intervals to determine the dynamics of the water-induced egg diameter increase. These data are shown in Figure 4.

Channel catfish unfertilized eggs reach maximum swelling about 12 minutes after water addition (Figure 4), while in 0.15 M NaCl egg swelling occurs over a several hour time course (Figure 3). Chorion elevation occurred in all groups including those held in Hank's salts at both temperatures (Figure 5). However, the chorions in the water group showed smooth contours while the Hank's salts groups all showed delamination (d) of the chorion with a rough outer contour (Figure 6). Subsequently, unfertilized eggs began ooplasmic contractions starting at 3 hours for water, 75% and 100% Hank's, but not beginning until 5.5 hour for 125% Hank's at 28°C. All eggs at 2°C, regardless of treatment, failed to begin ooplasmic movements throughout the incubation.

Objective 3. *Develop techniques to identify, assess and improve gamete quality.*

Objective 3a. *Develop criteria for standardizing and classifying egg quality prior to injection and after manual stripping and describe the morphological and physiological condition of channel catfish eggs including evaluation of morphological changes of oocytes during oocyte maturation in female catfish.*

University of Memphis. Initial images of catfish oocytes and embryos were made by automated transparency scanners. Automated transparency scanners imaged catfish oocytes and embryos during oocyte maturation and embryogenesis, respectively. This technology was developed for analysis of motility mutants in zebrafish (Computer-Aided-Screening, CAS) and is being adapted for analysis of

Figure 3. Channel catfish unfertilized egg and chorion diameter after addition of water (top panels), 0.15 M NaCl (middle panels) or 0.15 M NaCl and 10 mg/ml ovalbumin (bottom panels). Imaged with transparency scanner at 1200 dpi, resulting images were stacked and diameters were measured using Image J; 20 eggs were measured for each panel (duplicate wells) and presented as the mean +/- SEM.

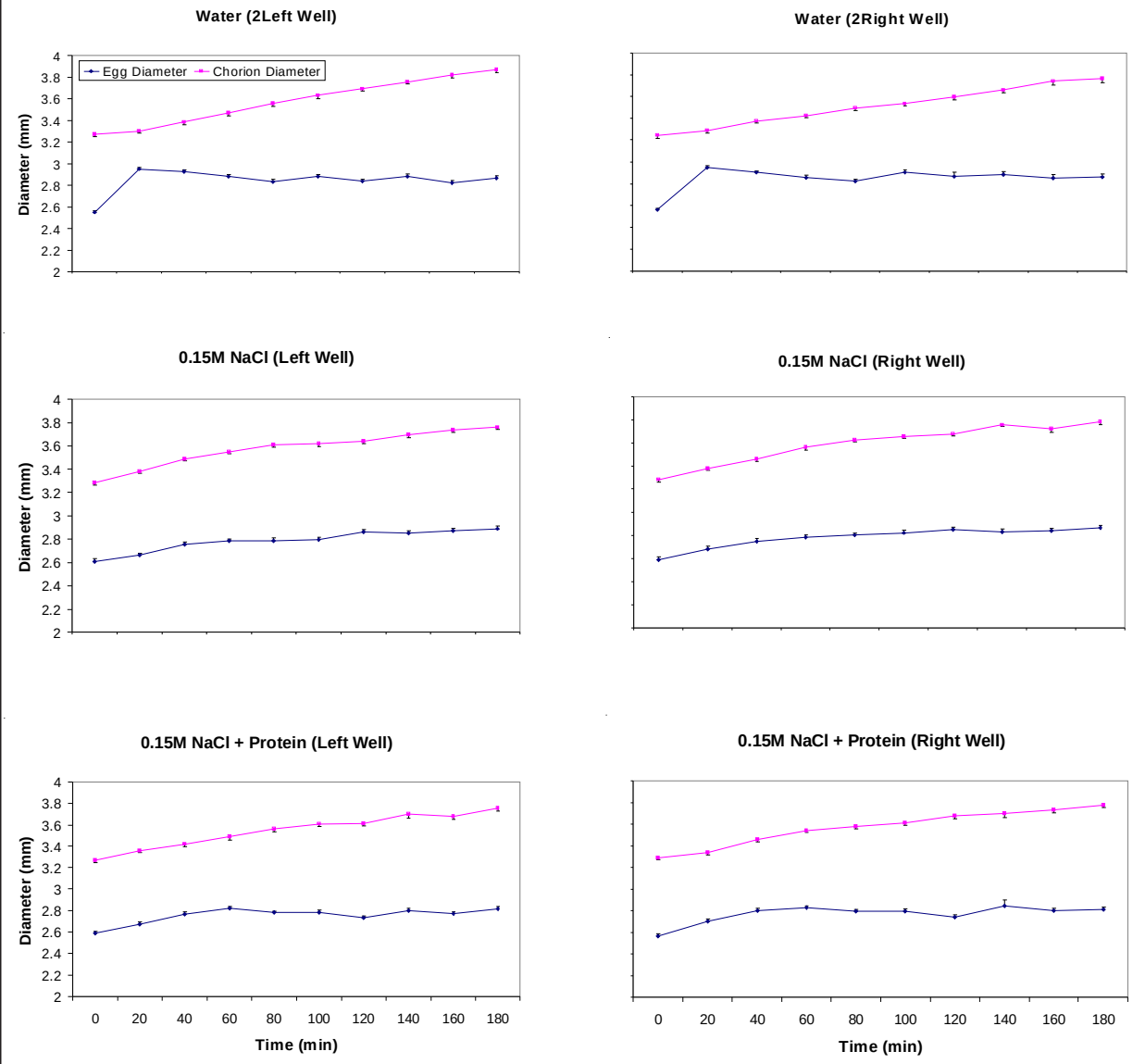


Figure 4. Channel catfish unfertilized egg diameter after water addition. Imaged with a transparency scanner every 2 min, the resulting images were stacked and analyzed with ImageJ. Data are presented as mean +/- SEM (n = 20 per time point). Data set from same group as upper left panel of Figure 3.

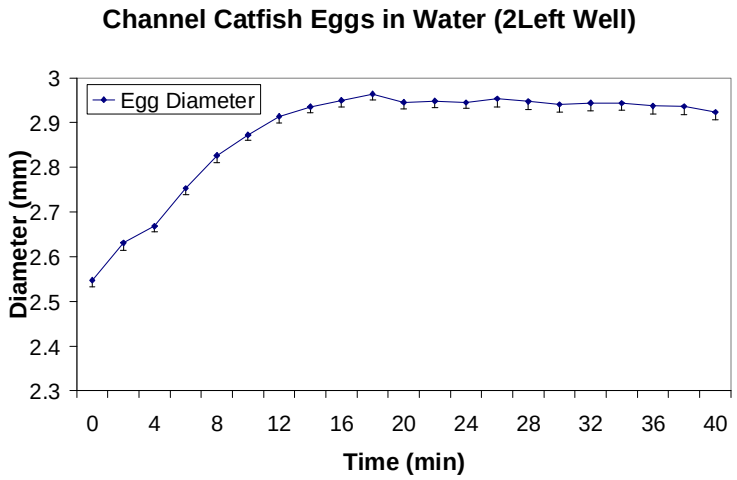


Figure 5. Montages of channel catfish unfertilized eggs after addition of water, 75%, 100% or 125% Hank's salts solution and imaged with transparency scanners; at left 28°C and at right 2°C.

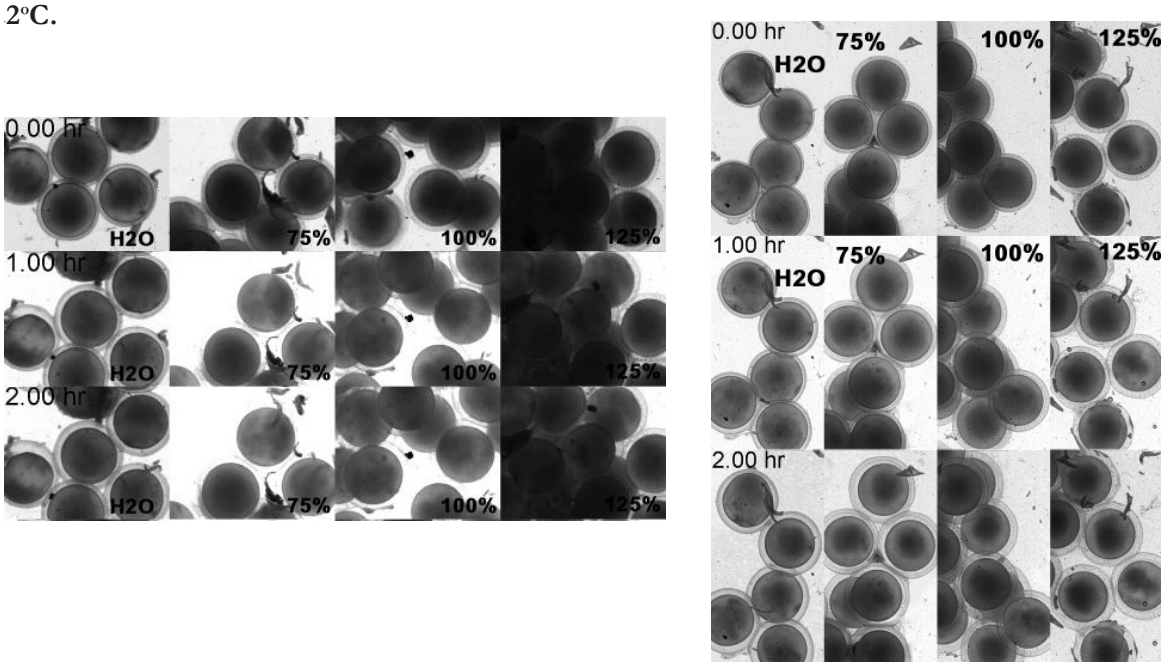
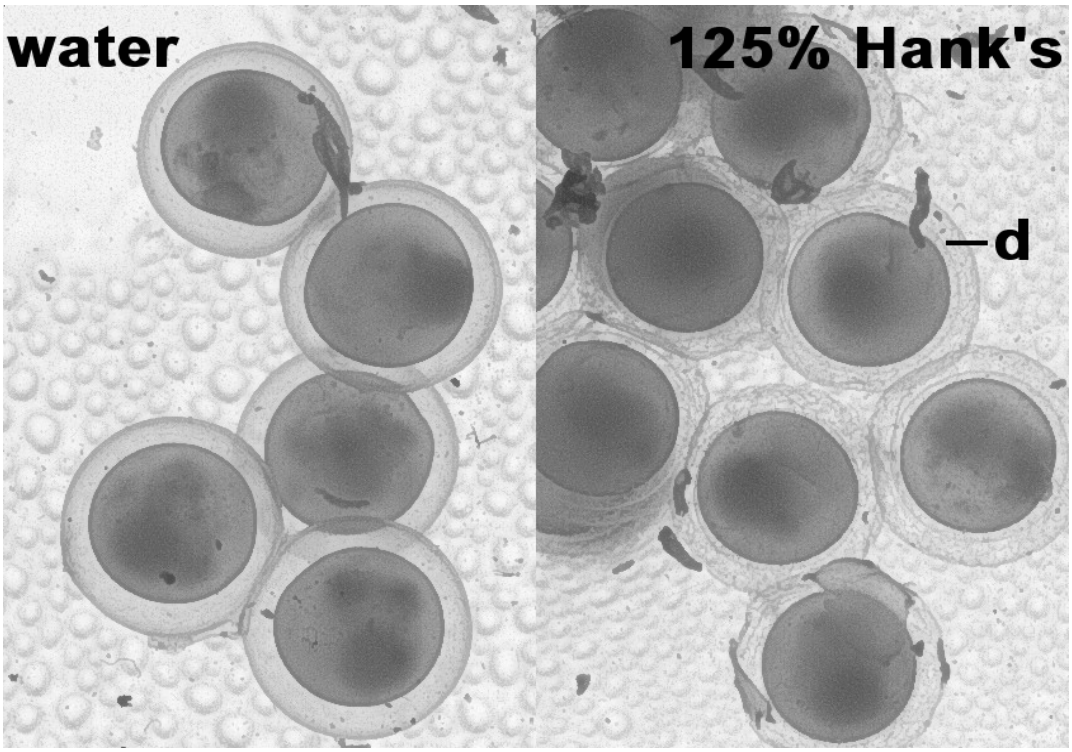


Figure 6. Comparison of chorions of water vs. 125% Hank's salts treated unfertilized channel catfish eggs after 4hr. Water activated eggs have swollen chorions that have smooth outlines and clear perivitelline spaces (left panel) by contrast, Hank's treated eggs have swollen chorions that have rough outlines and obvious delaminations (d) obscuring the perivitelline spaces (right panel). Egg diameters are noticeably larger in the water vs. Hank's group at this time (4hr). Water activated eggs have begun ooplasmic movements while those treated with Hank's have not.



catfish oocytes and embryos. Initial trials indicate that CAS may be used to follow catfish embryos throughout their 6- to 7-day period of development to hatching. The CAS system worked quite well in spite of the prolonged development time for catfish embryos (6 to 7 days compared with 2 days for zebrafish).

Animations of time-lapse image stacks in ImageJ revealed a surprising amount of cell movement in cleavage stage embryos. Other details of embryonic development included gastrulation/epiboly, neurulation, initiation of motility and hatching. Arrested development and subsequent cytolysis of abnormal embryos could also be clearly documented, including the developmental events prior to arrest and death.

In collaboration with Dr. Terry Tiersch at LSU, broodfish in ponds were subjected to elevated water temperatures early in the year in an attempt to induce early gonadal maturation and spawning in 2005. Eggs were stripped from female channel catfish and fertilized from sperm obtained from blue catfish testes. Observations were made on development and survival of the progeny produced.

Several hybrid and channel spawns were obtained and imaged by Computer-Aided Screening (CAS).

The initial analysis of two parallel runs (trials 1 and 2) showed a time window of mortality that corresponded to approximately 2000 to 2900 minutes post-fertilization (33 to 48 hours post-fertilization). Initial analysis of the CAS images revealed embryos undergoing cytolysis as expected (Figures 7 and 8). However, upon closer examination, development was arrested in some embryos and they failed to gastrulate, yet they continued to survive. Cleavage-

Figure 7. Viability of hybrid catfish and channel catfish embryos (trial 1) using CAS.

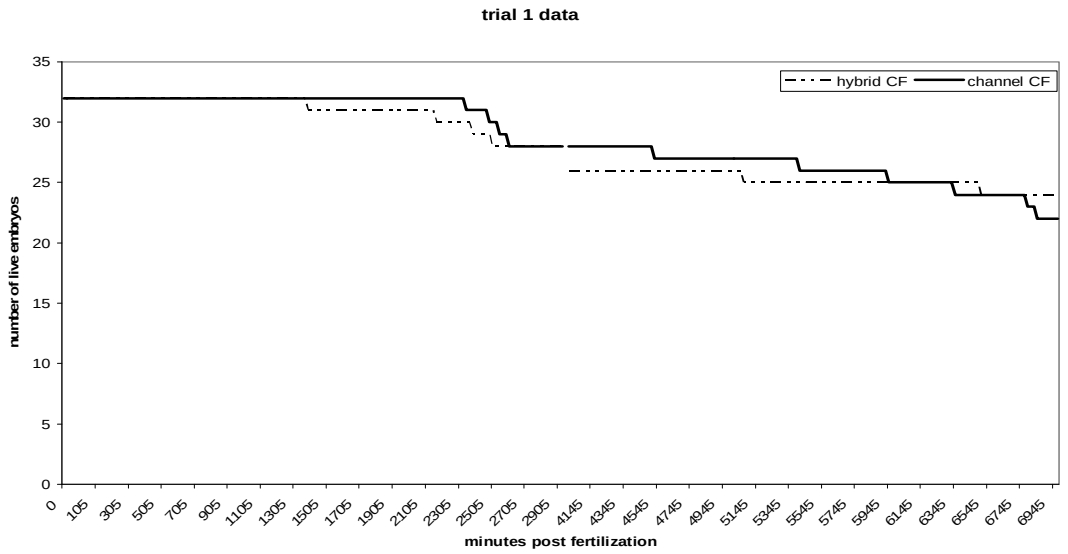
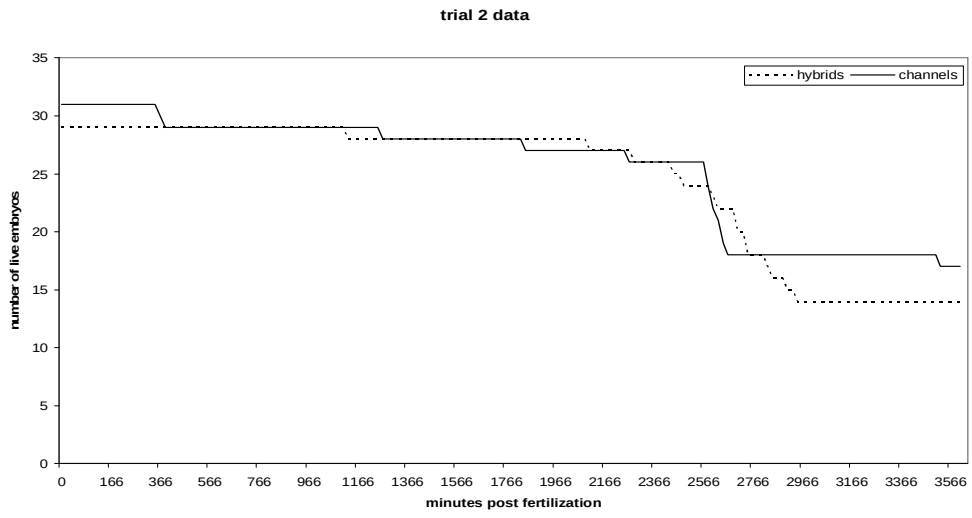


Figure 8. Viability of hybrid catfish and channel catfish embryos (trial 2) using CAS.



arrested embryos continued to show these movements in spite of failed development. Developmental arrest is not necessarily followed immediately by cytolysis and death. We are currently examining this surprising finding in more detail. Lambert, Small and Chatakondi have also observed this window of critical development, and treatment of embryos at this developmental stage is discussed in Objective 4. During this same time period, mortality occurs in channel catfish embryos exposed to antisense contructs designed to disrupt dorsal-lateral orientation. The cause of this developmental arrest needs to be ascertained and corrected.

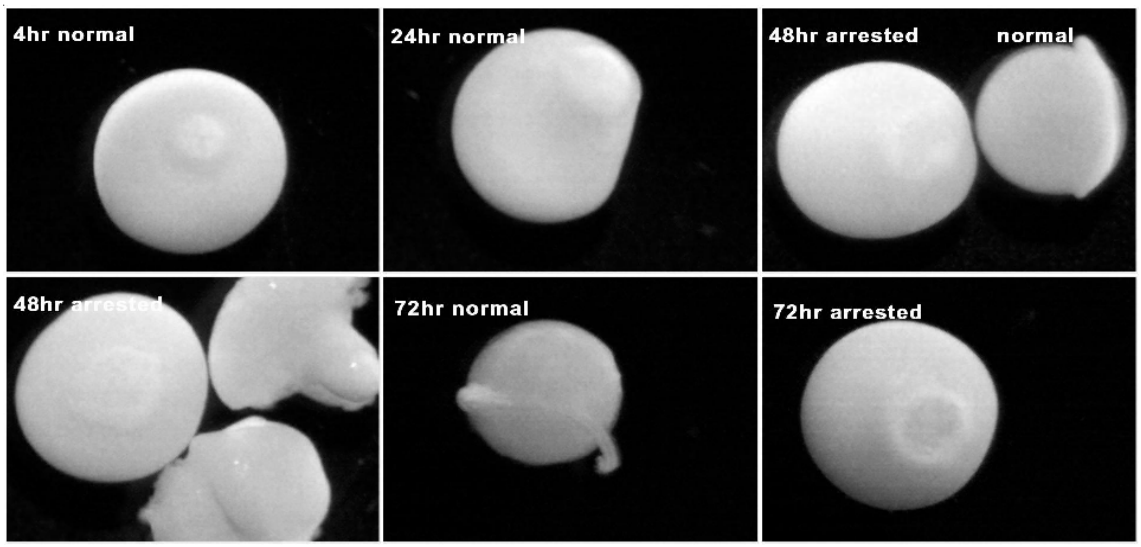
Embryo densities of 30 or more per well were found to be deleterious to embryos imaged by the CAS system even with flows greater than 10 mL/hour. While embryo densities of 16 or less embryos per well allowed complete development to hatch in the CAS system.

In addition to cytolysis, developmentally-arrested embryos may continue to survive and superficially appear normal and viable. However, careful imaging of groups of hybrid embryos at various times after fertilization revealed cleavage stage embryos persisting until at least 72 hours post-fertilization (Figure 9). This surprising result may explain some of the variability observed in hatch rates for hybrid catfish embryos.

Large ovarian follicles were obtained from a single channel female and were tested for germinal vesicle (GV) position and response to progestogen. Application of 5% acetic acid elicited clearing of the ooplasm and visualization of the GV. The dynamics of the clearing process was determined and imaged using CAS (Figure 10). GV identity was verified by presence of numerous nucleoli upon microscopic examination (Figure 11).

Additional images of catfish oocytes and embryos were made by automated transparency scanners. Daily use of 20-FL 4% formalin per well provided

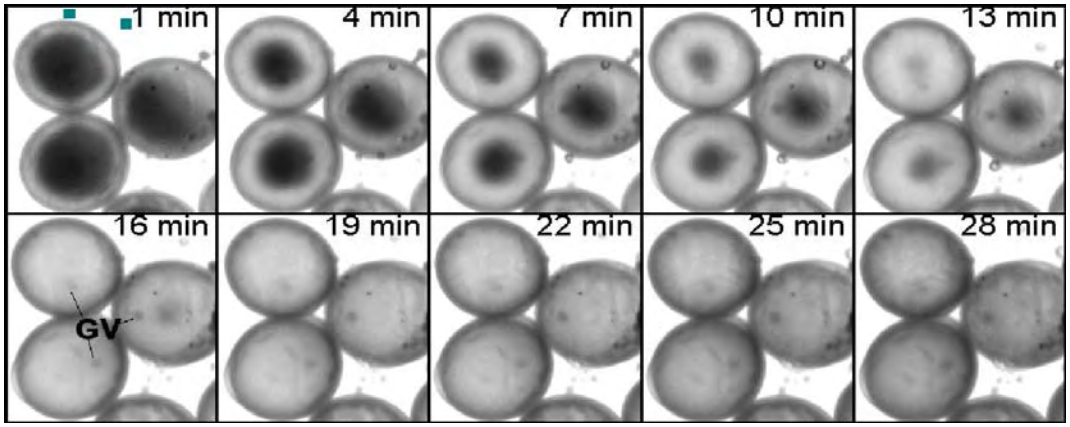
Figure 9. Digital photomicrographs of dechorionated, formalin-fixed hybrid catfish embryos. Embryos may arrest development in the cleavage stage but not immediately cytolyze.



protection from parasitic infestations that prevented complete development of catfish embryos within the CAS system previously. We have begun high resolution imaging of fixed embryos to develop an embryo staging table for channel and hybrid catfish.

A test of 0.5% bovine serum albumen (BSA) as an egg extender in Hank's salts was found to be less effective than Hank's salts alone for hybrid catfish from the USDA-ARS Stoneville, Mississippi, facility. The CAS system worked quite well in spite of the prolonged development time (6 to 7 days).

Figure 10. Channel catfish oocytes imaged by CAMMA and treated with 5% acetic acid. This treatment clears the yolky ooplasm and reveals the presence and location of the oocyte nucleus or germinal vesicle (GV).



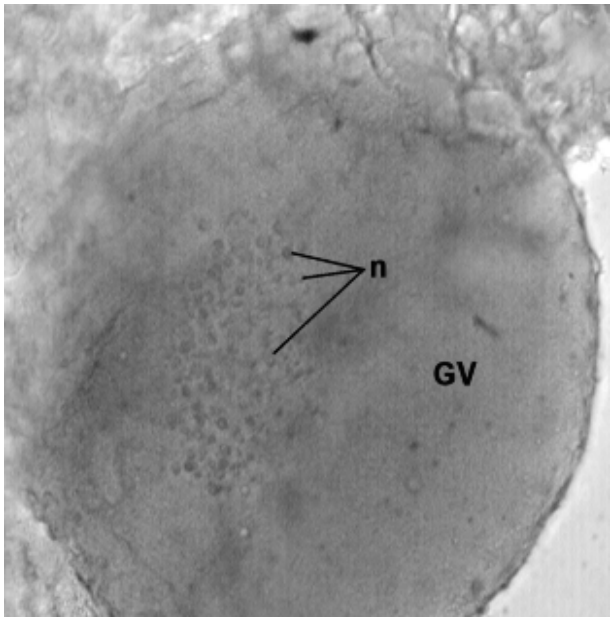


Figure 11. Isolated channel catfish oocyte germinal vesicle (GV) after 5% acetic acid fixation. Note the large number of characteristic nucleoli (n), a definitive set of landmarks for the GV.

Cytoplasmic movement and hence viability may occur without complete or continued embryonic development in both hybrid and channel catfish embryos at least for hours to days. We wanted to determine 1) if unfertilized, but water activated, channel eggs produced these cytoplasmic movements and 2) to what extent cleavage occurred in the absence of sperm. Channel catfish eggs from brood females at the USDA Catfish Genetics Research Unit, Stoneville, Mississippi, were obtained and divided into several batches. Some batches were placed directly into well water to activate the eggs without sperm. Other batches were placed into Hank's balanced saline solution and subsequently fertilized with blue catfish sperm or activated with water alone. Computer aided screening (CAS) was used to follow changes in eggs and embryos over time. Activated, but unfertilized, eggs showed the characteristic movements seen previously in normally fertilized embryos. In addition, blastodisc enlargement and protrusion also took place mimicking normal development (Figure 12). None of the activated, unfertilized eggs underwent gastrulation. To determine if these eggs did in fact undergo cleavage, groups were fixed in 4% formalin, dehydrated in methanol and stained with DAPI to visualize DNA and cleavage furrows, as described previously for fertilized eggs. No nuclei or cleavage furrows were evident compared to normally fertilized eggs prepared in the same way (Figure 13).

Results at a glance...

- *Water activated, but unfertilized, eggs showed the characteristic movements seen previously in normally fertilized embryos. Blastodisc enlargement and protrusion also took place mimicking normal development, however, none of the activated, unfertilized eggs underwent gastrulation or cleavage.*

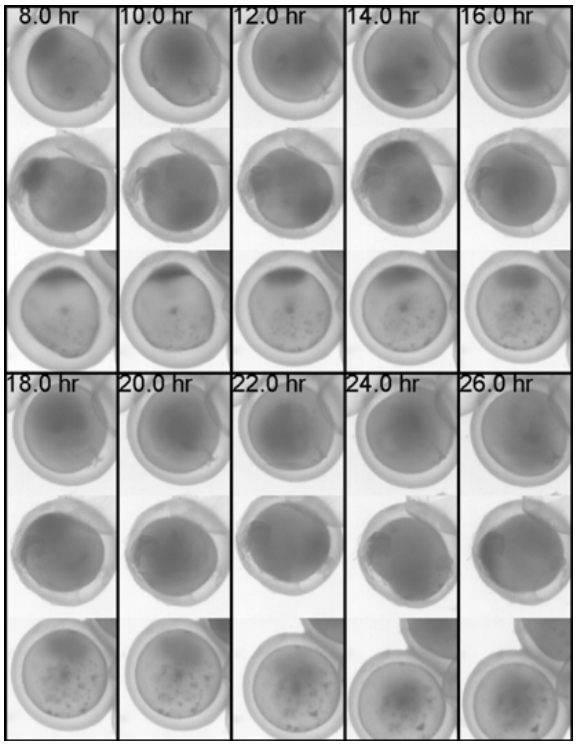


Figure 12. Three representative activated, unfertilized channel eggs imaged by CAS. Time after water addition shown at the top of each panel. Note the dark blastodisc found at one end of each egg in the top, left panel. Also note the change in egg outline indicating cytoplasmic movements or contractions. None of these underwent gastrulation.

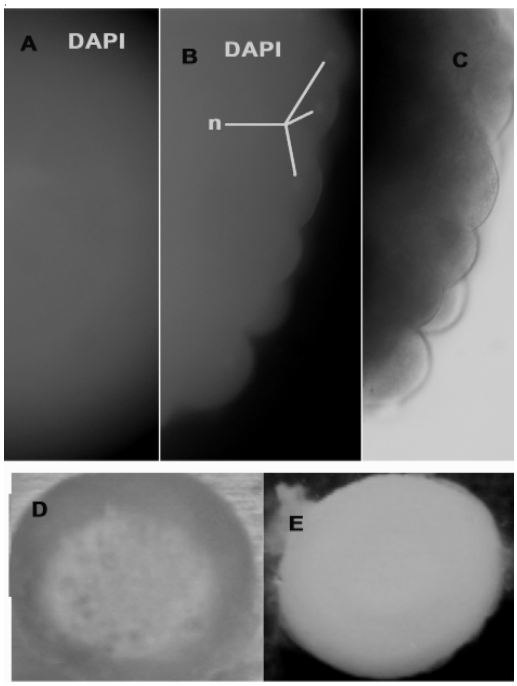


Figure 13. Comparison of activated, unfertilized channel eggs (A & D) to hybrid catfish embryos B, C & E) at 5.5hr post activation or insemination, respectively. DAPI DNA staining revealed blastomere nuclei (n) in fertilized egg blastodisc (B) but not in activated, unfertilized egg blastodisc (A). Brightfield (C) shows rounded blastomeres with prominent cleavage furrows in the fertilized egg. The corresponding dissecting microscope view (E) of the fertilized egg shows a uniform blastodisc with a faint halo of clear ooplasm, by contrast, the blastodisc of the activated, unfertilized egg (D) seems riddled with crater-like holes and lacks a halo around the irregular blastodisc.

To determine the effect of Hank's balanced saline solution on subsequent early development of hybrid catfish embryos, eggs from two channel females were fertilized with blue catfish sperm after eggs had no pre-incubation, 2, 3 or 4 hours of Hank's saline treatment. The results of initial scoring for blastodiscs containing normal and abnormal cleaving blastomeres (Figure 14) is shown in Table 38.

Figure 14. Comparison of normal (B) and abnormal (A) cleavage stage hybrid catfish embryos. Embryos were fixed in 4% formalin, dehydrated in MeOH and manually dechorionated to allow direct inspection of the cleaving blastodisc. Normal embryos have a smooth blastodisc with a regular outline, often with an apparent halo of clear ooplasm. Abnormal embryos have an irregular, pock-marked blastodisc without the halo.

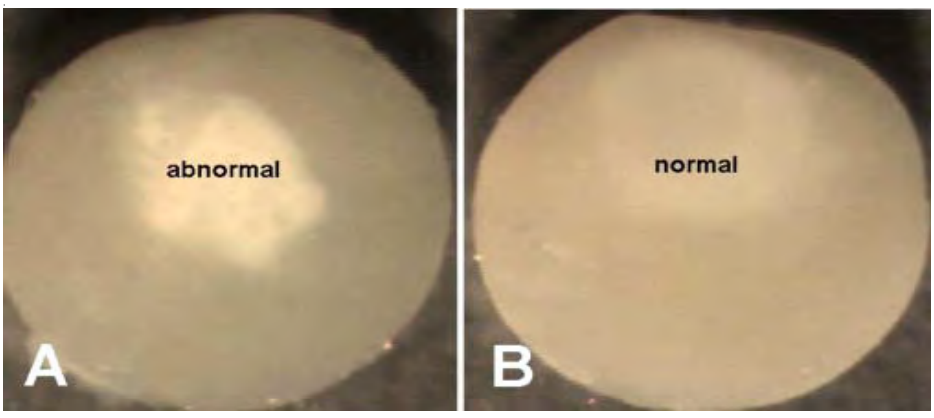


Table 38. Effect of channel egg incubation in Hank's balanced salt solution on development to early and late cleavage stages after insemination with blue catfish sperm. Data presented as number of embryos fixed and manually dechorionated presenting smooth blastodiscs with halos of clear ooplasm (scored as normal), all others were deemed abnormal or arrested.

	Hanks hrs	5/25/06 6pm normal	early cleavage total	% normal	5/26/06 10am normal	late cleavage total	% normal
female 1	0	8	11	73	2	7	29
	2	1	8	13	5	10	50
	3	10	16	63	1	11	9
	4	5	10	50	2	10	20
female 2	0	8	14	57	4	10	40
	2	12	14	86	nd	nd	nd
	3	11	12	92	7	10	70
	4	13	15	87	8	13	62

The results presented in Table 38 indicate that channel eggs remain fertilizable in Hank's balanced saline for at least 4 hours and can develop at least through late cleavage. Nevertheless, there does seem to be a decrease in development from early to late cleavage in these samples, suggesting some progressive developmental arrest.

Since the results presented in Table 38 indicated that channel catfish eggs may remain viable and fertilizable for an extended period, eggs incubated for 24 hours in Hank's were imaged using CAS while either remaining in Hank's or after being transferred to fresh, dechlorinated water (activation conditions). The results (Figure 15) indicate that eggs in Hank's after 24 hours are already undergoing cytoplasmic contractions, albeit reduced, and that activation by fresh water did not affect the egg significantly. This surprising result suggests that refinement of a saline based on Hank's saline may allow short-term (hours to days) storage of eggs, thereby expanding possible husbandry practices with hybrids.

We are currently extracting data from stacks of scanned images from 1) catfish oocytes treated with various media and hormones, and 2) analysis of embryonic development. We also plan to screen catfish oocyte and ovary extracts for reaction with cell-cycle control protein anti-bodies (e.g., anti-cyclin B1) that may prove useful in our studies of oocyte maturation in catfish. We are carrying out large scale dechorionations and imaging of the samples fixed at various times post insemination, especially comparing the early developmental time course of channel × channel, blue sperm × channel (hybrids), and unfertilized, but activated channel eggs. In addition, these samples will compare eggs pre-incubated in Hank's saline versus eggs used directly from the female.

Louisiana State University. Ultrasound is a user-friendly technology capable of creating ultra-clear images that can be captured as movies or still images. Ultrasound has been used extensively in human medicine and livestock species, but has had limited application in finfish. This non-invasive technique has been used with female livestock to monitor follicular growth through ovulation. It has also been used as a tool for sex identification and

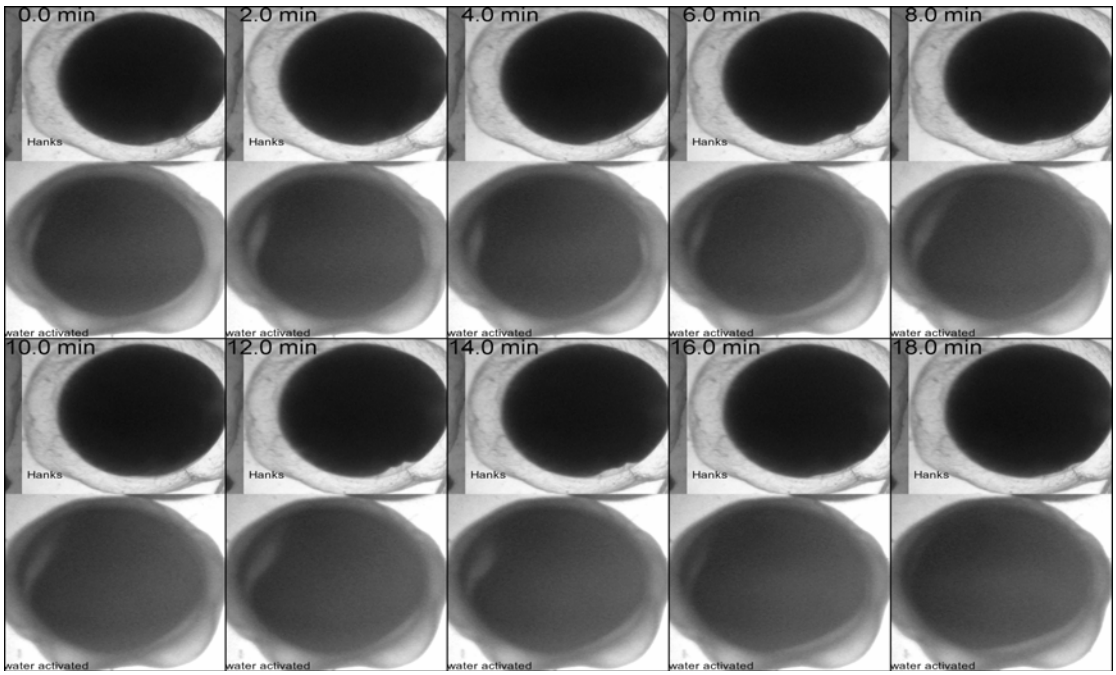
carcass evaluation in several species of fish (e.g., Atlantic salmon, Atlantic halibut, striped bass, shovelnose sturgeon, and barfin flounder). The objectives of this study were to evaluate visibility of gonads at different life stages, ovarian development in strip spawned and non-spawning females, oocyte diameter, compare ultrasound measurements to physical measurements, classify females prior to hormone injection, identify the time to strip eggs after injection, and determine the efficacy of stripping by use of ultrasound in channel catfish.

During February through June, 2004 channel catfish gonads were evaluated at three different life stages: fingerlings (under 0.4 kg), market-sized food fish (0.4 to 0.8 kg), and brood stock (more than 1.5 kg). Fish were scanned using a linear ultrasound probe (3 to 10 mHz), and gonadal sex was verified by dissection. To evaluate ovarian development, 12 females were given injections of artificial luteinizing hormone-releasing hormone. Of these, five were

Results at a glance...

- *Ultrasound may be used to ascertain ovulation in channel catfish females and the appropriate time for stripping of eggs. Spectrophotometric assays can be used to determine sperm concentrations from crushed testis of catfish. Utilization of these tools should result in more efficient use of sperm and more consistent fertilization rates.*

Figure 15. Channel catfish eggs stored in Hank's saline for 24 hours then imaged with CAS. Images were taken every 2 minutes (at top of each panel). The egg at the top (darker) remains in Hank's saline, note the contractions of the egg cortex from one time slice to the next. The water activated egg (lighter) is somewhat swollen (hydrated) and shows similar cortical contractions.



strip-spawned. Fish were scanned daily to monitor gonadal development.

Gonads were correctly identified as testis or ovary for fingerlings (57%), food fish (90%), and brood stock (86%). Immature gonads were difficult to distinguish from surrounding tissues. Mature testes were partially visible, but we could not quantify their development due to lack of contrast with surrounding tissues. Unlike testes, mature ovaries were easily distinguished and their development quantified by measuring ovarian diameter, calculating the ratio of ovarian diameter to body wall diameter (OD:BD), and measuring oocyte diameter. There were no significant differences in ovarian diameter or in OD:BD between strip-spawned and non-spawning females (Table 39). Strip-spawned females had significantly larger oocyte diameters than non-spawning females on days 3 and 4 after injection. The results indicate that ultrasonography could be a useful tool for monitoring ovarian development in channel catfish. This could be used in artificial spawning of large groups of females, such as in production of hybrids of channel catfish females and males of blue catfish.

During January to June of 2005, 234 channel catfish females were scanned in situ using a 3 to 10 mHz linear probe on a laptop ultrasound (TELAVET 1000, Classic Medical, Tequesta, Florida). Ultrasound measurements were compared with physical caliper measurements of the body cavity and oocytes diameters for 15 female catfish. Conditioned females (N = 210) were seined from ponds, scanned by ultrasound, and classified as "already spawned," "poor ovarian development," or "good ovarian development." Seventy-two females (6 sets of 12) classified as having "good ovarian development" were given injections of luteinizing hormone-releasing hormone analog (Peninsula Laboratories Inc., San Carlos, California). Females were scanned at 2 to 8 hour intervals to identify the proper time to strip eggs. After the fish were stripped, scanning was repeated to determine the efficacy of stripping.

Ultrasound measurements were not significantly different from the physical measurements (Table 40). With ultrasound, a trained technician could classify fish prior to injection within 10 seconds. Identifying the time to strip females involved several factors including a decrease in the space between the body cavity and the ovaries and an increase in ovarian edema (black areas) that were coincidental to ovulation of eggs into the lumen of the ovary.

Using these criteria, a trained technician could identify when to strip eggs during induced spawning by classification of eggs as "under-ripe," "ripe" and "overripe." After fish were stripped, ultrasound

Table 39. Ovarian and oocyte development of strip-spawned (N = 5) and non-spawning (N = 7) females after hormone injection. Daily means for strip-spawned and non-spawning fish within each variable that share letters were not significantly different (P<0.01).

	Ovarian Diameter (mm)		OD:BD*		Oocyte Diameter (mm)	
Day	Strip-spawned	Non-spawning	Strip-spawned	Non-spawning	Strip-spawned	Non-spawning
1	54.7 ± 5.8a	54.2 ± 7.4a	0.87 ± 0.03a	0.84 ± 0.05a	1.8 ± 0.4a	1.8 ± 0.5a
2	65.8 ± 5.1a	50.4 ± 9.1a	0.89 ± 0.03a	0.85 ± 0.04a	1.9 ± 0.4a	1.9 ± 0.5a
3	70.6 ± 6.6a	63.9 ± 7.4a	0.89 ± 0.03a	0.88 ± 0.05a	2.2 ± 0.5a	1.8 ± 0.4b
4	60.6 ± 0.0a	64.3 ± 11.3a	0.90 ± 0.00a	0.87 ± 0.06a	2.0 ± 0.4a	1.8 ± 0.5b

*Ovarian diameter : Body wall diameter

Table 40. Ultrasound and physical diameter measurements (mean ± SE) for channel catfish females (N=15). Means sharing a letter within each variable were not significantly different (*P*>0.05).

Diameter	Ultrasound measurement (mm)	Physical measurement (mm)	% Difference *
Body cavity	70.4 ± 1.0a	68.4 ± 2.0a	-3
Oocyte	1.7 ± 0.1a	1.9 ± 0.1a	9

*[(Physical measurement – Ultrasound measurement)/ Physical measurement] x 100

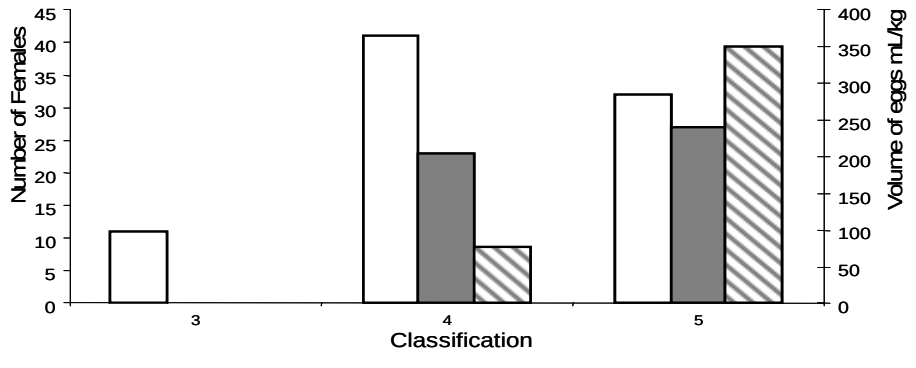
determined if a second stripping was necessary. The ability to identify proper timing of collection of ripe eggs from females would increase efficiency of hybrid catfish production by improving egg quality and possibly increasing numbers of fry produced.

During 2006, ultrasound was tested on a production scale. The goal of this study was to evaluate ultrasonography as a non-invasive production tool to improve the efficiency of artificial spawning in channel catfish females. The objectives were to use ultrasound to: 1) classify females prior to hormone injection; 2) identify the time after injection to strip eggs; and 3) determine the efficacy of stripping.

Between April and May 2006, approximately 800 channel catfish females were scanned in situ using a 3-10 mHz linear probe on a laptop ultrasound (TELAVET 1000, Classic Medical, Tequesta, Florida) at Baxter Land Company (Watson, Arkansas). Females were classified before injection on a scale of 1 to 5, with 5 indicating full ovarian maturity. Fish classified as 3 (fair), 4 (good), or 5 (excellent) (N = 84) were injected with carp pituitary extract, and were scanned periodically to identify the proper time to strip eggs. After the fish were stripped (N = 50), scanning was repeated to determine the efficacy of stripping.

For the three classifications of fish that were injected, there was a significant difference in the percent of females that released eggs and in the quantity of eggs produced by the females (Figure 16). A trained

Figure 16. Ultrasound was used to classify females as fair (3), good (4) or excellent (5). Selected fish were injected (white bars), stripped (gray bars), and the volumn of eggs was recorded (crosshatched bars).



technician identified the period when stimulated females could be stripped with 79% accuracy. Ultrasound techniques were able to determine the effectiveness of the stripping procedure. Because of the increase in the percentage of females spawned and the quantity of eggs produced per kilogram of female, ultrasound classification of females prior to injection could be used as a tool to increase the efficiency of hybrid catfish production.

In 2007, commercial scale studies continued at Baxter Land Company in Desha County, Arkansas. The objectives were to evaluate the use of ultrasound to: 1) screen female brood stock for hormone injection; 2) evaluate spawning success of selected female brood stock; 3) estimate the number of eggs produced by stripped females, and 4) determine the average time taken to scan each fish.

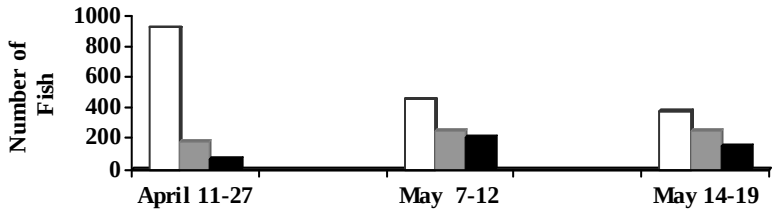
Three sets of female broodfish were evaluated in April and May of 2007 using a 3-10 mHz linear probe on a laptop ultrasound. Ovarian development was categorized as minimal (1); underdeveloped (2); fair (3); good (4); or excellent (5). Fish exhibiting minimal and underdeveloped ovaries were not injected. Selected females were injected with carp pituitary extract. The priming (2 mg/kg) and resolving (8 mg/kg) doses were administered 14 hours apart. Eggs produced by stripped females were fertilized with blue catfish sperm. Egg volumes collected per female were recorded.

The application of ultrasound in a commercial setting improved the selection of females for hormone injection in three trials (Figure 17). A total of 1,635 female broodfish were scanned via ultrasound, and 42% were selected for hormone injection. More than 6 million eggs were produced, with an average of 5,328 eggs per kg body weight of female spawned. Spawning results from April were influenced by system management and disease. Differences in the spawning rates in the two trials in May may be due to differences in the experience levels of ultrasound technician. Overall, ultrasound scans averaged 2 to 3 fish per minute.

Auburn University. The variation in the timing of egg collection and the act of manually collecting eggs may impact the quantity and quality of eggs obtained. The ovulation process and factors that might affect egg quality was examined.

Female channel catfish were held individually in aquaria and induced to spawn using LHRHa at 120 µg/kg body weight. Fish were monitored hourly as ovulation approached. The time of the first egg release into the aquaria was noted. Fish releasing eggs were randomly assigned to one of four treatments: stripped at 1 hour post-egg release, sacrificed at 1 hour and the ovary collected, stripped at 4 hours post-egg release, and sacrificed at 4 hours and the ovary collected. An additional set of injected females were paired with male channel catfish and

Figure 17. Fish were scanned using ultrasound (white bars), selected for injection (gray bars) and resulted in 34%, 81% and 61% spawning rates (black bars) in three trials.



allowed to spawn naturally. Data on egg quantity and quality was collected for each female including number of eggs/g of eggs, egg diameter, fecundity and viability 48 hours post-fertilization. Samples of un-ovulated eggs and ovulated eggs from fish manually stripped at different times were collected and frozen for biochemical analyses.

The gonadosomatic index (GSI) index of females at the time of first egg release averaged 21.31%. The time of stripping after the first egg release had little effect on the quantity or quality of eggs obtained. Fish stripped at approximately 2 hours after the first egg release had a mean fecundity of 6,374 ± 2,111 eggs/kg compared to fish stripped at 4 hours of 7,086 ± 3,330 eggs/kg. The number of eggs/g egg mass (42.5 ± 9.4 and 41.1 ± 12.9) were the same for the two times of stripping groups. Egg diameter averaged 3.51 mm for both groups. Likewise egg viability at 48 hours post-fertilization did not differ between strip times, 85.1 ± 12.6 and 87.0 ± 9.4 respectively. No differences were seen in the characteristics of the first set of eggs stripped from a female and the last set of eggs stripped. Fecundity of same-age females induced to spawn and either manually stripped or which spawned naturally were similar, 7,804 ± 2,954 and 6,166 ± 4,570, respectively.

Proper selection of broodfish for induced spawning can help insure a high rate of spawning success and good egg quality. However, the brood selection is often subjective based on general appearance of the fish and the biologist's experience. Appropriate quantitative criteria can reduce individual bias and assists the less experienced biologist in brood selection. In 2005, five trials were conducted using 3- and 5-year-old channel catfish females where the physical characteristics of total length, weight, and width were measured and ratios calculated and egg samples taken for fatty acid profiles. Percent lipids of eggs collected in 2005 were compared. Eggs collected during the first stripping had lower percent lipids than those collected during the third stripping, 4.34 ± 1.28 and 5.59 ± 0.76 respectively. The time at which eggs were collected post-ovulation had some effect on % lipids with eggs collected at 1 hour after egg release having 4.28 ± 1.57 % lipid and those collected at greater than 4 hours having 5.03 ± 1.25%. Three-year-old and 5-year-old females had similar percent lipids in ovulated eggs. Fatty acid profile of the above egg samples has been analyzed and the results are being processed.

A study was conducted in the 2006 season to determine if testis development and sperm characteristics were related to external morphology. Rio Grande strain male blue catfish were fed for 6 months either a non-enriched commercial catfish diet or an enriched diet where docosahexaenoic acid and arachidonic acid were added. All males were described as to their weight gain, body proportions (length, head width and girth) and photographed. GSI, relative percentage of anterior testis, sperm count and motility time were recorded for each sacrificed male.

Testicular development (GSI) was not related to body weight, body length to head width ratio or body length to head girth ratio. Likewise, relative percent of anterior testis was not related to the above characteristics. There was no apparent relationship between body weight, body length to head width ratio or body length to head girth ratio to sperm count or sperm motility.

When 26 pairs of channel catfish female × blue catfish male were allowed to spawn naturally in pens in ponds, two spawns were obtained (7.7%). When 13 pairs of channel catfish female × channel catfish male were allowed to spawn naturally, three spawns were obtained (23.1%). The number of eggs/kg obtained and egg viability at 48 hours after fertilization was similar for channel male and blue male spawns, again indicating that there is no genetic incompatibility between male blue catfish and female channel catfish. When low hatch rates are obtained for hybrid embryos, brood stock management, gamete quality and hatchery management are the problem, not species incompatibilities.

Objective 3b. *Determine the profile of estradiol hormone from serum plasma of 2-year-old females of channel catfish over a 12-month period, determine changes in oocyte maturation during vitellogenesis and identify the different cathepsins that are responsible for vitellogenin degradation and oocyte maturation in female catfish.*

Mississippi State University. The catfish industry is hampered by a chronic inefficiency resulting from the low spawning success of female brood stock for the annual production of fingerlings. Current estimates of spawning success of females range from 20 to 30%. An understanding of the relationship of annual changes in physiological indices during a reproductive cycle to oocyte maturation and successful spawning in channel catfish may contribute to an accurate prediction of successful spawns. The objective of this study was to evaluate the effects of plasma steriod concentrations (estradiol and testosterone), egg size and protein degredation by cathepsins D, L and B on in vivo egg maturation in four strains of channel catfish.

The first study of profiles of plasma estradiol and testosterone concentrations, size and protein content of eggs in 2- to 3-year-old channel catfish has been completed for four commercial strains of channel catfish, Gold Kist (2 strains), Thompson and NWAC103 for one year (age 2 to age 3). This study also included the first measurements of the activities of the proteolytic activities of cathepsins D, L, and B and their relationships to other processes involved in oocyte maturation. All of the parameters collectively evaluated may serve to assist in the selection of the best 2-year-old channel catfish female brood stock, and to determine the optimal timing of treatments of hormone injection to increase reproductive performance.

Groups of nine, 2-year-old female channel catfish brood stock obtained from each of four different strains/sources were tagged and stocked into four 0.1-acre earthen ponds (36 fish per pond, 9 fish per strain) in April. Blood and egg samples were collected from 12 fish in each pond (3 fish/strain) every month for 11 and 9 months, respectively, for blood and eggs. No individual fish within a strain was subject to sampling more than once every four months.

Great variation among individuals of the same strain precluded the identification of any significant, strain-specific differences for the variables under investigation. For all strains, mean plasma estradiol concentrations ranged from 0.02 to 0.29 ng/mL from June through December, and increased dramatically in January, peaking in February (3.4 to 3.7 ng/mL), and remained above 1.00 ng/mL through May.

Mean plasma testosterone concentrations increased from May through September (0.03 to 1.23 ng/mL), decreased in October, and then increased and remained at approximately 1 ng/mL through April. When variables from fish of all strains were collectively evaluated over time, concentrations of both plasma estradiol and testosterone significantly increased in July and again later from February to May. The increase in hormone concentration was accompanied by oocyte growth and increases in proteolytic activity of specific cathepsins, supporting the role of estradiol in regulating vitellogenesis.

During oocyte development, there were sequential relationships among hormone concentration, cathepsin activity, protein content, and predominant oocyte proteins. Plasma levels of vitellogenin gradually increased from February and peaked in May. Vitellogenin was enzymatically broken down into smaller protein units by cathepsins L, D, and B that individually predominated during different months when different stages of oocyte development occurred.

Mean activities of cathepsins D and L steadily

increased beginning in October and were highest in March, whereas the activity of cathepsin B was variable from month to month. High levels of activity of cathepsin L occurred during February and March, suggesting its important role in protein degradation during that time, while peak activity of cathepsin B occurred during November to January. Activities of cathepsin D were the highest recorded, peaking in March, April, and May. Cathepsin B is more important in oogenesis or early vitellogenesis, cathepsin L assumes a principal role during middle vitellogenesis, and activity of cathepsin D peaks during late vitellogenesis.

Mean protein content of eggs was highest in October (3.08 to 3.795) when eggs appeared and decreased to levels of 0.54% to 2.14% for the remainder of the year (November through April) when eggs were present. From October to November the mean egg size increased by approximately 40%, to 1.0 to 1.4 mm, and remained at this size until May and June when size increased by approximately 75% to 100%.

Twenty hours subsequent to the injection of fish with either carp pituitary hormone or luteinizing hormone releasing hormone, plasma estradiol and testosterone concentration increased, activities of cathepsins L, D, and B increased, and egg size and protein content increased. These changes stimulated oocyte maturation. The percentages of spawning obtained were 18.8% of LHRH-injected fish, 12.4% of CPE injected fish, 9.4% of fish not injected, and 0% of saline injected fish.

Injection of females with LHRHa can potentially serve as a tool to increase spawning success in appropriate commercial settings, particularly for improving 3-year-old catfish spawning success early in the spawning season. Low levels of plasma estradiol in all 3-year-old fish suggest that insufficient stimulation of vitellogenin production by estradiol may underlie the lack of vitellogenin incorporation into developing oocytes. Sufficiently high peaks in estradiol concentration in July likely indicate a reproductively mature female. This information should serve as a foundation to apply in the evaluation of the relative effectiveness of exogenous hormone treatments in increasing the spawning success of channel catfish for producing both intraspecific and interspecific embryos.

Objective 3c. *Develop in vitro assays to evaluate sperm quality and evaluate their predictive ability in relation to fertilization and hatch.*

Louisiana State University. Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hank's balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1 g/20 mL) to release sperm. The sperm solutions were poured through a 100-µm filter into a 50-mL conical tube. Sperm motility was estimated after activation with deionized water and concentrations were calculated using duplicate hemacytometer counts. Optical density of the sperm solutions was measured using absorbance readings obtained by spectrophotometry (Spectronic 20 Genesys) at wavelengths of 400, 450, 500, 550 and 600 nm.

The most accurate absorbance readings for determining sperm concentrations from whole testis occurred at 500 nm ($y = 2^9 + 1.99$, $R^2 = 0.531$). These results indicate that spectrophotometric assays can be used to determine sperm concentrations from crushed testis of channel catfish.

Flow cytometery is commonly used to evaluate cells

from a wide variety of species. We have applied this technology to assess sperm quality in catfish. Several assays are being developed including evaluation of plasma membrane integrity, determination of the ratio of sperm cells to somatic cells, and evaluation of mitochondrial function. Studies were performed this year using sperm subjected to a variety of treatments including refrigerated storage, cryoprotectant toxicity, freezing, and ultraviolet irradiation. The data from these studies are being analyzed and further studies are underway to develop practical methods to use flow cytometery as a tool for evaluation of catfish sperm.

Objective 4. *Develop economically viable standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.*

Objective 4a. *Determine optimal sperm (fresh, frozen and refrigerated)-to-egg ratios for fertilization and hatch.*

Louisiana State University. The production of hybrid catfish fry is limited by factors including the inefficient use of sperm from the male blue catfish. The time, effort and expense involved in rearing a blue catfish male to maturity requires efficient use of the sperm obtained when the male is killed. The objectives of this study were to evaluate the effects of concentration on refrigerated and cryopreserved blue catfish sperm for: 1) sperm motility, 2) fertilization of channel catfish eggs, and 3) hatch of hybrid fry.

In 2004, only channel catfish males were available, and they were used for experimentation. Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hank's balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1g: 20 mL) to release sperm. The sperm solutions were poured through a 100-µm filter into a 50-mL conical tube. Sperm motility was estimated after activation with de-ionized water and concentrations were calculated using duplicate hemacytometer counts. The solutions were diluted to contain 1×10^8, 1×10^7 and 1×10^6 sperm cells/mL and were used for fertilization during artificial spawning with eggs from two females and sperm from three males (0.5 mL/400 eggs). The sperm concentration of 1×10^6 yielded 71 ± 16% fertilization for fresh sperm (3 ± 5% for thawed sperm); 1×10^7 yielded 88 ± 9% fertilization for fresh sperm (45 ± 37% for thawed), and 1×10^8 yielded 91 ± 10% fertilization for fresh sperm (48 ± 55% for thawed). The varied concentration of sperm used for artificial spawning yielded significant differences in fertilization and there is a correlation between sperm concentration and fertilization.

Results at a glance...

- *Sperm concentrations can be reduced in currently used fertilization protocols by 100-fold with little reduction in subsequent hatch rate. This should result in much more efficient use of male broodfish when producing hybrid catfish.*

Six sexually mature male blue catfish were killed and their testes were surgically removed during the 2005 spawning season (May and June). The testes were cleaned of excess blood and tissue, weighed and

placed in Ziploc bags containing 1:10 (testis weight:volume of extender) Hank's balanced salt solution prepared without calcium and magnesium. The testes were crushed and poured through a 100-μm filter. Sperm motility was estimated and concentrations of the samples were determined using hemacytometer counts. The initial sperm dilutions were divided into three groups. The first group was used as a control at the original concentration ($3.6\times10^8 \pm 4.6\times10^7$ cells/mL). The remainder was diluted to final concentrations of 1×10^8, 1×10^7 and 1×10^6 cells/mL. The control and diluted samples were divided into two aliquots; one was cryopreserved at Genex Custom Collections, Inc., and the other was stored in a refrigerator (4°C) until used for fertilization. Eggs were stripped from gravid female channel catfish that had been injected with 100 μg/kg of synthetic LHRHa for artificial spawning. A monolayer of eggs was poured into 100-mL cups and fertilized using either one 0.5-mL straw of cryopreserved sperm or 0.5 mL of refrigerated sperm at the three concentrations. The fertilized eggs were placed into cups in a hatching trough for incubation. Neurulation was used as a conservative measurement of fertilization and was estimated in all cups at 24 hours after fertilization. The number of hatched fry was recorded at 120 hours after fertilization.

There was a significant difference in motility across the various concentrations and between refrigerated and cryopreserved sperm. In addition, there was a significant difference in neurulation, although there was no significant difference in hatch across the various concentrations (Table 41). Given that current hatchery practice is to use sperm dilutions prepared around 1:10 (weight:volume), this study suggests that refrigerated and cryopreserved blue catfish sperm can be diluted considerably (100 times greater) without reducing fertilization of channel catfish eggs.

Table 41. Mean neurulation and hatch rate for channel catfish × blue catfish hybrid embryos fertilized with either refrigerated or cryopreserved sperm at undiluted (approximately 4×10^8, 1×10^8, 1×10^7, or 1×10^6 sperm/400 eggs. Data for refrigerated and cryopreserved sperm treatments were pooled as no significant differences were found between these two sperm preparations. Means within a column sharing a letter were not significantly different.

	Mean ± SD	
Sperm/400 eggs	Neurulation	Hatch
Control	70 ± 14a	55 ± 17a
1×10^8	67 ± 18ab	58 ± 16a
1×10^7	64 ± 16ab	51 ± 21a
1×10^6	61 ± 11b	47 ± 15a

Objective 4b. *Determine the effects of commonly used therapeutics on hatching success.*

USDA-ARS. The chemotherapeutic and respective concentration yielding the greatest hybrid hatching success was identified. Four hybrid catfish egg masses were each divided into thirteen equal sub-masses. Each sub-mass was subjected to once daily chemotherapeutic treatment as a 15-minute static bath until eyed. The treatments were as follows: (1) Control (no treatment), (2) 125 ppm hydrogen peroxide, (3) 250 ppm hydrogen peroxide, (4) 500 ppm hydrogen peroxide, (5) 50 ppm formalin,

(6) 100 ppm formalin, (7) 200 ppm formalin, (8) 50 ppm povidone iodine, (9) 100 ppm povidone iodine, (10) 200 ppm povidone iodine, (11) 2.5 ppm copper sulfate, (12) 5 ppm copper sulfate, and (13) 10 ppm copper sulfate. Egg masses were allowed to hatch to completion within individual containers. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was calculated as the percentage of eggs hatched.

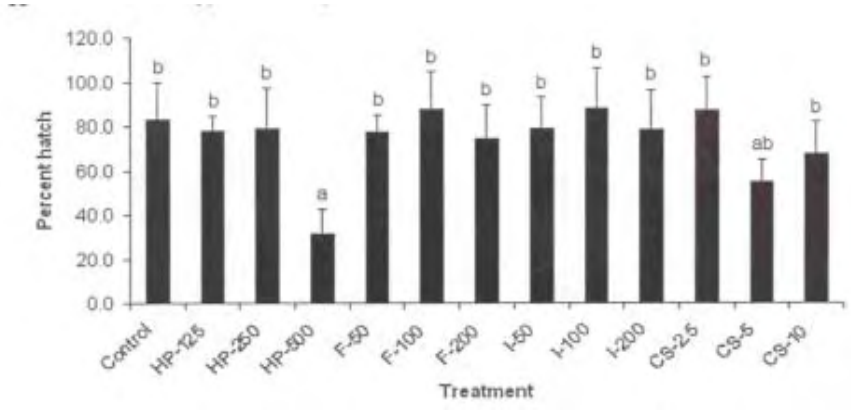
Results at a glance...

- *The frequency of formalin treatments should be three per day to maximize hatch rate of hybrid embryos. Four treatments per day is excessive. At 28°C, hybrid embryos are chemically sensitive to formalin between 42 to 46 hours post-fertilization, and formalin treatments should be avoided during this period.*

Hatching success was high in the untreated controls (82.8%) and highly variable within treatments. Overall, hatching success was not significantly improved with chemo-therapeutic treatments; however, a tendency toward increased hatching success was observed among eggs treated with 100 ppm formalin (87.7%), 100 ppm iodine (88.1%), and 2.5 ppm copper sulfate (87.0%). A significant decrease in percent hatch was observed in eggs treated with 500 ppm hydrogen peroxide (Figure 18).

The optimal treatment frequency for maximizing hybrid hatching success was determined. Formalin is the most common therapeutant used to treat catfish egg diseases, and formalin yielded one of the highest hatching success rates in the first experiment. For these reasons, formalin was chosen as the therapeutant for this experiment. Four trials were conducted with four egg masses per trial to determine the optimal frequency of formalin application for maximizing hatching success. Formalin treatments were administered 0, 2, 3, or 4 times daily as a 100 ppm static bath. Egg masses were allowed to hatch to completion within individual containers. When hatching was complete, the fry were siphoned into

Figure 18. Mean hatching success (%) of hybrid catfish eggs treated daily with increasing doses of hydrogen peroxide (HP), formalin (F), povidone iodine (I), or copper sulfate (CS). Numerals refer to dosage rate (ppm). Bars with common letters are not statistically different.



a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was calculated as the percentage of eggs hatched. The optimal frequency of formalin treatments was determined to be three times daily (Table 42).

The effect of withholding formalin treatment during a putative sensitive developmental stage on hybrid hatching success was determined. A preliminary study was conducted to ascertain the developmental stage at which mortality most often occurs in hybrids. Briefly, hybrid eggs were collected throughout development, cleared in Stockard's solution and microscopically elevated for developmental differences indicative of egg mortality. At 28°C, mortality was observed between 42 and 46 hours post-fertilization. To determine the effect of withholding treatments during this potentially sensitive developmental period, formalin treatments (100 ppm) were administered three times daily such that treatments occurred at 42 hours post-fertilization (control) or were withheld from 42 to 44, 42 to 46, or 42 to 48 hours post-fertilization. Hatching success was calculated as previously described. Formalin treatments administered at 42 hours post-fertilization significantly reduced hatching success. Withholding treatments until 46 hours post-fertilization at 28°C yielded the greatest percent hatch (Table 43).

Table 42. Effect of daily formalin treatment frequency on hybrid hatching success.

	Frequency of daily formalin treatments			
	0x	2x	3x	4x
Percent hatch	12.7 ± 4.5a	31.4 ± 4.6b	51.6 ± 3.6c	33.7 ± 4.6b

Means followed by different letters are statistically different (P<0.05).

Table 43. Effect of formalin treatments administered at 42 hours post-fertilization (control) or withheld from 42 to 44, 42 to 46, or 42 to 48 hours post-fertilization on hybrid hatching success at 28ºC.

	Time of formalin treatment (hours post- fertilization)			
	42 h	44 h	46 h	48 h
Percent hatch	19.6 ± 5.3a	30.7 ± 11.0b	58.3 ± 3.9c	34.1 ± 8.5b

Means followed by different letters are statistically different (P<0.05).

WORK PLANNED

All participants are working on data analysis and reports. The University of Memphis reported results for evaluating extenders for eggs. These experiments were not part of the original work plan, and are being conducted in addition to the original work planned. They are closely related to the objective for storing sperm, and constitute a new objective in this area.

IMPACT

At the beginning of this project, only about 4 to 5 million hybrid catfish fry were being hatched. Research results from this project have been important in increasing hybrid catfish production to more than 25 million fry hatched in 2006. This figure increased to 32 million fry hatched in 2007, and we know of 4 farms actively engaged in producing hybrid fry. Three of these locations are planning further expansion.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications

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