REALIZED HERITABILITY AND RESPONSE TO SELECTION FOR FECUNDITY, HATCHING RATE AND FRY/KG FOR CHANNEL CATFISH FEMALES (*Ictalurus punctatus*) INDUCED TO OVULATE AND FERTILIZED WITH BLUE CATFISH (*Ictalurus furcatus*) MALES FOR THE PRODUCTION OF HYBRID CATFISH EMBRYOS

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Megan E. Gima

Certificate of Approval:

_____	_____
Zhanjiang Liu Professor Fisheries and Allied Aquacultures	Rex A. Dunham, Chair Professor Fisheries and Allied Aquacultures
_____	_____
Nagaraj Chatakondi Affiliate Professor Fisheries and Allied Aquacultures	George T. Flowers Dean Graduate School

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Megan E. Gima

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Megan E. Gima

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Signature of Author

_____
Date of Graduation

VITA

Megan Elizabeth Gima was born on November 12, 1982 in Indianapolis, Indiana. Megan is the youngest child of Jerry and Colleen McDaniel. She grew up in Bunker Hill, Indiana with her brother and sister, Joseph Rosinski and Kristi Rabe. Megan graduated from Purdue University of West Lafayette, Indiana in 2006 and entered the graduate school of Auburn University in May of 2006. She married Andrew Gima in 2008.

THESIS ABSTRACT

REALIZED HERITABILITY AND RESPONSE TO SELECTION FOR FECUNDITY, HATCHING RATE AND FRY/KG FOR CHANNEL CATFISH FEMALES (*Ictalurus punctatus*) INDUCED TO OVULATE AND FERTILIZED WITH BLUE CATFISH (*Ictalurus furcatus*) MALES FOR THE PRODUCTION OF HYBRID CATFISH EMBRYOS

Megan E. Gima

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The objective of the this research was to determine the heritabilities and response to selection for fecundity, percent hatch and fry per kilogram body weight for channel catfish females induced to ovulate with luteinizing hormone releasing hormone analogue (LHRHa) for the production of hybrid catfish embryos.

Age and/or environmental effects influences the reproductive traits measured over two spawning seasons. When the fish were three years old the relative fecundity was 10,246 and 10,126 for the select and control lines, respectfully. The percent hatch and

fry per kilogram female for select line was 34.7 and 2,817 and for the control was 35.5 and 3,465, respectfully. Realized heritabilities were 0.10, -0.03, and 0.06 for fecundity, percent hatch and fry per kilogram female, respectively.

When the fish were four years old, the relative fecundity was 9,429 and 8,968 for the select and control lines, respectfully. The percent hatch and fry per kilogram female for the select progeny was 9.79 and 1,088 and the control progeny was 13.28 and 675, respectfully. Realized heritabilities were 0.42, -0.13, and -0.06 for fecundity, percent hatch and fry per kilogram female, respectively.

A response to selection for fecundity was observed for both years with realized heritability ranging from 0.10-0.42. This trait appears to have additive genetic variation and can be improved via selection. Based on the results from both years the heritability for percent hatch is zero. No additive genetic variation was found for percent hatch. A potential economically significant improvement in fry output of the three year old females was observed, although heritability was extremely low. There was no improvement in fry output when the fish were four years old. This subtle difference could be a result of a genotype X environment interaction, with the severe cold conditions masking any genetic differences. Alternatively, genotype X age effect may explain the change in results.

Additive genetic variation for channel catfish reproductive traits may be higher than indicated by the results of this study. Fry per kilogram female and percent hatch for the progeny control was five to six times higher than that of the parental generation control, thus the performance of the control was dramatically improved. This increase could be due to improvements in husbandry, an inadvertent selection response or both.

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INTRODUCTION

The farming or culturing of catfish had its start in Kansas in the 1930s and in Mississippi and Arkansas in the 1940s. Channel catfish, *Ictalurus punctatus*, dominates the United States aquaculture industry representing over 50 percent of the total aquaculture production (Dunham et al. 1998; Dunham et al. 1999, Liu et al. 2003; Engle 2003, Rezk et al. 2003). However, total production and sales are now declining, over the last seven years catfish production has seen a plateau and have decreased by one third (USDA 2000; Harvey 2005; USDA 2005, USDA 2008).

Average price per pound for foodfish size catfish for 2006 and 2007 in the United States was $0.78 and $0.75, respectively. The number of foodfish sized catfish sold in 2006 and 2007 was 370,872,000 and 352,651,000 fish respectively. The total sales for 2006 were $455,095,000 and for 2007 were $408,750,000. The number of fish for fingerling and fry size sales for 2006 was 572,434,000 and 2007 was 488,786,000 fish, with total sales of $21,315,000 and $20,103,000 respectively. The average per pound price for fingerling and fry was $1.70 in 2006 and was $1.86 in 2007 (USDA 2008). The total sales for catfish in the United States were $444,835,000 in 2007 (USDA 2008).

With improvements in aquaculture techniques, such as species, strain, line and family selection, spawning, incubation, and grow out, the production of catfish could be improved to counteract the adverse economic climate that is damaging the catfish industry today. Production could increase with the utilization of the interspecific hybrid

catfish produced by a channel catfish female and a blue catfish male, which out performs the traditionally cultured channel catfish for many traits.

The hybrid produced from the female channel catfish, *Ictalurus punctatus* and the male blue catfish, *Ictalurus furcatus*, is the best catfish for pond culture and has shown improvement for various culture traits as compared to the primarily culture species, channel catfish, thus making the hybrid catfish attractive to the aquaculture industry (Dunham et al. 2001; Chatakondi et al. 2004; Dunham et al. 2008). Heterosis for many traits has been demonstrated by the pairing of a channel catfish female and a blue catfish male (Dupree and Green 1969; Dupree et al. 1969; Dunham et al. 1982; Dunham and Smitherman 1983; Dunham et al. 2000). The hybrid catfish, when compared to channel catfish, shows positive differences in growth uniformity, growth rate (20-100%), feed conversion (an increase by 15-20%), dress-out percent and fillet percentage (5-10%), disease resistance and survival (10-100%), low dissolved oxygen tolerance, and success of harvest by seining (50-100%) therefore, the hybrid has more superior culture traits (Yant et al. 1975; Chappell 1979; Brooks et al. 1982a,b; Dunham et al. 1982; Dunham et al. 1983; Ella 1984; Dunham 1987, Dunham et al. 1990; Jeppsen 1995; Wolters et al. 1996; Dunham and Argue 1998; Dunham and Brummett 1999; Chatakondi et al. 2000; Li et. al 2004).

Large scale application of the hybrid in the catfish industry has been constricted by insufficient production of hybrid catfish fry (Tave and Smitherman 1982; Goudie et al. 1993; Dunham et al. 1998; Dunham et al. 1999; Dunham et al. 2000; Argue et al. 2003; Kristanto 2004). Significant progress has been made in improving technology for producing hybrid catfish embryos (Dunham et al. 2004; Chatakondi et al. 2005). Despite

the significant, culture trait improvements, the production technology to produce the hybrid catfish embryo, needs further improvements before utilization of the hybrid is widespread through out the United States. Improvements to fry production has been made through improved nutritional preparation, temperature manipulation, spawning protocols and hormone induction for channel catfish females and improved fungal control on egg masses. One area that has not been well researched or documented in fishes is the improvement in reproductive traits through genetic improvements. Selection to improve reproductive performance in channel catfish females may increase reproductive success for the production of hybrid catfish embryos.

Reproductive traits are highly heritable and often respond to selection in fish (Tave 1993). Selection for reproductive traits has already been proven to have high heritabilities in a variety of cultured species. Acros et al. (2004) found that heritabilities for reproductive traits, such as days to first spawn after ablation, egg number, and egg diameter indicate selection for these traits could improve reproductive output for the female shrimp, *Penaeus (Litopenaeus) vannamei*. In another study, heritabilities for days to spawn, egg number and proportion hatched were 0.47±0.15, 0.41±0.18 and 0.18±0.16, respectively, for black tiger prawn, *Penaeus monodon* (Macbeth et al. 2007).

Gall and Gross (1978), studied reproductive performance in rainbow trout, including volume of eggs spawned, egg size, egg number and fertility to the eyed egg stage. Differences among stocks were attributed to both genetic and environmental effects. Heritability estimated for these traits ranged from 0.05 to 0.76 for individual stocks. When the stocks were averaged together the h^2 was much higher and ranged from 0.32 to 0.52 (Gall and Gross 1978). In another rainbow trout study by Gall and Huang

(1988), heritability estimates, using the sire components of variance, ranged from 0.15 to 0.32 for reproductive traits. Based on these estimates, selection response should be about 15% per generation if combined family and within-family selection of females and sib selection of males was conducted (Gall and Huang 1988).

Recently, Gall and Neira (2004) found high estimated heritabilities for weight of green eggs spawned (0.39) and number of green eggs (0.42), medium heritabilities for average egg weight/ egg size (0.32) and number of eyed eggs (0.33), and a moderate heritability estimate for spawn day (0.24) in farmed coho salmon. This indicated that the study population had high levels of additive genetic variation for female reproductive traits (Gall and Neira 2004).

Although selection or heritability experiments for reproductive traits have not been published for channel catfish, genetic variation is present. Broussard and Stickney (1981) observed large strain differences for reproductive traits of channel catfish. Channel catfish strains spawn at variable ages with no significant difference in the success of spawning (Broussard and Stickney 1981; Dunham et al. 1983). Additionally, large differences were found in the production of hybrid fry per kilogram in strains of channel catfish females (Ballenger 2007).

If catfish hybrid embryo production and related reproductive traits are heritable, channel catfish females could be selected to increase hybrid fry output. This would lead to a greater availability of hybrid catfish fingerlings to improve production and efficiency in the catfish industry. The objectives of this research were to determine the heritabilities and response to selection for fecundity, hatching rate and fry/kg for channel catfish

females induced to ovulate with luteinizing hormone releasing hormone analogue (LHRHa) for the production of hybrid catfish embryos.

MATERIALS AND METHODS

Experimental Fish: Parent generation

The channel catfish females, channel catfish males and the blue catfish males were maintained at the E. W. Shell Fisheries Research Center, Alabama Agricultural Experiment Station, Auburn University. In May of 2003, 77 female channel catfish were hormone induced to ovulate with LHRHa. The resulting eggs were fertilized with either blue catfish or channel catfish sperm to produce hybrids or channel catfish. Individual spawns were kept separate. Fecundity, hatch and fry/kg were measured for each female. Channel catfish fry from females with average production were pooled and those from the top performing 25% of the females were pooled, and then stocked in ponds to serve as the controls and select line, respectively. These fish were harvested in March of 2005 and females separated from males. Fish from select and control lines were marked with either a pit tag or heat branded or both and then stocked into communal ponds.

The female channel catfish were harvested again and stocked in two 0.1 hectare ponds on September 19, 2005. One pond was initially stocked with 156 females and the other with 213 females. Females were a mix of AU lines (Ballenger 2007).

The channel catfish male brood stock were initially stocked into a 0.1 hectare pond with 120 males on September 20, 2005. The channel catfish males used for the channel egg masses were corresponded to the same channel catfish female lines.

The blue catfish male brood stock were initially stocked into a 0.1 hectare pond. The blue males used for the hybrid eggs masses were a mixture of RG, 197, TBB, and D&B lines. Means for reproductive traits of select and control parental lines are found in Table 1.

Experimental Fish: Progeny generation

A total of two spawning trials or runs were conducted during the 2006 spawning season. The first trial was initiated by implanting the three- year- old females with LHRHa on May 30 and 31, 2006. The water temperature was 29.4 and 29.2°C, respectfully, at the time when females were implanted. A second run was initiated on June 14 and 16, 2006. At the time the females were implanted the water temperature was 27.2 and 27.9°C, respectfully. A total of 68 control females and 151 select line females were utilized for both run.

The channel catfish females were harvested two days prior to the expected dates of ovulation by seining the pond. The females were selected to receive hormone implants based on secondary sexual characteristics such as well-rounded, distended abdomen, a reddened or swollen urogenital area, or darkened pigmentation (Bart et al. 1998; Dunham et al. 1998; Dunham et al. 1999; Kristanto 2004). Females not selected for spawning were returned to ponds potentially for the next spawning run or season. The females that were selected for the spawning trial were identified, weighed, given an individual female number and placed into a laundry bag. The bag was labeled with the female's individual number, line and weight. The bagged females were suspended on the side of troughs using clothes pins until the time of implanting with luetinizing hormone-releasing hormone analog (LHRHa) implants.

Table 1 Heritability and response to selection for fry per kilogram female, percent hatch, and fecundity correlated responses to selection for mean percent ovulation, latency period, percent hatch and relative fecundity for channel catfish (*Ictalurus punctatus*) females selected for increased fry per kilogram female when induced to ovulate with luetinizing hormone releasing hormone agonist and fertilized with channel catfish or blue catfish (*I.furcatus*) sperm

Year	Line	N (Female)	Percent Ovulation	N	Latency	N	Fecundity	N	Fry per Kilogram Female	N	Percent Hatch
	Select Parent	-	-	-	-	18	11,152±2,519	18	3,828±3,029	18	34.0±18.9
	Control Parent	-	-	-	-	30	9,904±1,604	30	577±465	30	5.9±4.7
	Selection Differential	-	-	-	-	-	1,248	-	3,251	-	28.1
2006	Select Progeny	101	93.1	94	51.0[a]±11.82	94	10,247[a]±3,421	94	3,311[a]±3,324	94	34.7[a]±0.3
2006	Control Progeny	115	87.0	99	50.1[a]±10.44	100	10,127[a]±3,535	100	3,124[a]±2,809	96	35.5[a]±0.2
	Response	-	-	-	-	-	120	-	187	-	-0.8
	h^2	-	-	-	-	-	0.10	-	0.06	-	-0.03
2007	Select Progeny	21	90.5	19	56.6[a]±7.87	19	9,429[a]±1,946	19	835[a]±1,833	19	9.8[a]±0.2
2007	Control Progeny	35	85.7	30	59.8[a]±12.81	30	8,910[a]±2,332	30	1,026[a]±1,636	29	13.3[a]±0.2
	Response	-	-	-	-	-	519	-	-191	-	-3.5
	h^2	-	-	-	-	-	0.42	-	-0.06	-	-0.13

The channel catfish males were harvested using a seine, approximately one day prior to the estimated ovulation of the females selected. The males were selected by secondary sexual characteristics such as darkened pigmentation; a well-developed, extended genital papilla; large, muscular pads on the dorsal surface of a broad head; also the presence of scraps or cuts on or around the head region indicating aggressive behavior towards other males. This aggressive behavior before their natural spawning season is a sign that the male has actively been actively fighting for spawning areas within the pond thus indicating the male's hormone profile is increasing or peaking. Non-selected males were returned to the pond for future use.

The blue catfish male brood stock were harvested one day prior to the estimated ovulation date of the selected channel females. The blue catfish were selected based on secondary sexual characteristics. Fish with a well developed genital papilla, a large, muscular head, and signs of aggression towards other males were selected for the trial. Males that were not selected for the spawning trial were returned to the pond.

The experimental fish, collection and design for the 2007 season was similar to the 2006 season. Only one spawning run was initiated by implanting the females with LHRHa on May 12, 2007.

Female channel catfish were initially stocked in on June 24, 2005. The four-year- old females were mix of AU lines and harvested on May 11, 2007. A total of 39 select line and 9 control line females were utilized for the 2007 spawning run.

Channel catfish male brood stocks were initially stocked in on May 17, 2005. The four year old channel males used for the straight channel egg masses were corresponded to the same channel catfish female lines and were harvested on May 11,

2007. The channel catfish male and female brood stocks were communally stocked into a 0.1 hectare pond with 160 fish total.

Blue catfish male brood stocks were initially stocked into twelve 0.1 hectare ponds with an average of 19.5±2.5 males on January 11, 2007. The five year old blue males used for the hybrid egg masses were RG, RG x D&B, D&B x RG, and Mix2/3 lines.

Administration of Hormone Implants

The channel catfish females were implanted with Reproboost ® Implants, an LHRHa implant. The precut implants were obtained from the Center of Marine Biotechnology, Columbus Center, in Baltimore, Maryland, USA. The implant was administered at a rate of 100µg per kilogram female. The implants prepared were preformed in varying sizes from 150 to 350µg. A 14 gauge hypodermic needle was used to place the implant in the intramuscular tissue located directly posterior to the dorsal fin and ventrally down the body an estimated 3cm (Hutson 2006). The females were then returned back to the holding tanks that were approximately 3.0m X 0.47m X 0.61m with a capacity of 837 liters of water.

Male and Sperm Preparation

Channel and blue catfish males were sacrificed within 24 hours prior to ovulation of females. Once they were sacrificed, a scalpel was used to cut the connective tissue attaching the testis to the abdominal cavity. Once the testes were removed, any existing connective tissue and granular testis (testicular area containing no viable sperm, located posterior to the viable sections) were carefully removed and discarded. The remaining testis were then washed with a saline solution (8.5g pickling salt/ L of distilled water) to

remove as much blood as possible. These testes were then gently dried with paper towel and weighed to the nearest 0.01 gram. Sections of the testis that had a red or opaque coloration were cut off and discarded. The remaining testes were white in color and appeared to be full of milt.

The milt filled portions of the testes were weighed to the nearest 0.01 gram. Saline solution, 8.5 ppt, was used to dilute the sperm at a ratio of 10ml saline solution/ gram usable testes. The testes were placed in a mesh strainer and maceration was accomplished by manually smashing the testes with fingers and allowing the sperm to drip into a weigh boat. The pre-measured saline was used to rinse the testicular tissue, fingers and the fine mesh strainer to ensure all milt was obtained. The saline, sperm solution was then mixed and poured into a centrifuge tube. Each male strain was identified, given an individual male number, and recorded. The labeled sperm solution was stored in a refrigerator at 5.0°C until needed for fertilization. Gibco ® Gentamicin Reagent Solution (Invitrogen Corporation, Grand Island, NY, USA) was added to channel sperm solution to maintain quality and freshness. The prepared sperm was swirled, gently, twice daily to maintain a homogenous solution.

Sperm density was determined using a spectrophotometer. A spectrophotometer set at a wavelength of 546 nm was used to estimate the density of the sperm in the solution. A small sample of the sperm solution was further diluted into a 9:1 ratio using 9 µL saline solution to 1 µL sperm solution. A sperm density graph was constructed by counting the number of sperm in the undiluted solution and a construct series of serial dilutions, determining the spectrophotometer reading, and regressing the series of serial

dilutions. Absorbance of actual sperm solution for fertilization was determined and plotted on the chart to determine sperm density.

Artificial Spawning

Starting 30 hours after the hormone was implanted; females were periodically checked for any eggs ovulated onto the mesh bag. When ovulation was detected, the bagged female was removed from the holding tank and placed in a tub containing a 200ppm tricaine methane sulfate (MS-222) solution. Sodium bicarbonate was also added to the anesthetic to buffer the solution at a ratio of 1:1. Once the opercula of the fish were significantly slowed and the body was limp, the female was removed from the solution and the mesh bag. The female was rinsed in freshwater and a towel was then used to remove any freshwater, so activation of the eggs prior to fertilization did not occur. The female's head was wrapped with the towel to prevent water dripping from the gills onto the eggs and to moist gills.

Weigh boats, aluminum or stainless steel pie pans were cleaned, dried, and lightly greased with vegetable shortening to prevent the stripped eggs from sticking to them. A small mass of eggs, approximately 30g, were stripped into a weigh boat and were designated to be fertilized with channel male sperm solution. Then the remaining eggs were stripped into the pie pans at approximately 200g per pie pan, until the female failed to express eggs. These larger egg masses were then later fertilized with blue catfish sperm solution. If blood or tissue was expelled with the eggs, saline solution was added to rinse the blood from the eggs. Blood clots, clumps of eggs or eggs still attached to ovarian tissue were manually removed to prevent the growth of fungus and data was

recorded. The cleaned eggs were then weighed to the nearest gram to calculate the approximate number of eggs from each egg mass and female.

Fertilization and Water Hardening

Eggs were fertilized by adding the appropriate amount of sperm solution to obtain a ratio of 6.5×10^7 sperm/ 100g of eggs, based on sperm density readings. A syringe was used to measure the appropriate amount of sperm solution and evenly add it to the eggs. The eggs and sperm solution were gently mixed and a small amount of fresh water was then added to just cover the eggs. The eggs, sperm solution and water were then gently mixed again to ensure all eggs have equal exposure to the sperm solution.

The eggs were allowed to sit undisturbed for two minutes, enabling the individual eggs to start forming one large egg mass. The eggs were then transferred to a flow through trough with fresh, pond water for 15-60 minutes. This allowed for further water hardening of the eggs and to maintain water temperature and water quality.

Incubation

Once the eggs water hardened, they were transferred to flow through paddle wheel troughs. The egg masses were taken out of the pie pans and placed in suspended mesh baskets within the paddle wheel troughs for incubation. The female and male number of each egg mass and the position of the egg mass within the trough were recorded to maintain the family identity.

Approximately 12 and 20 hours after fertilization formalin treatments (100ppm) were administered to the eggs (Small and Chatakondi 2006). Starting at 28 hours, 32ppm copper sulfate was added to each trough. After the initial copper sulfate treatment a treatment of copper sulfate in the morning, of formalin in the afternoon, and copper

sulfate again in the evening were administered daily at 8 hour intervals. Copper sulfate treatments were terminated shortly before the first eggs hatched. If fungus persisted within the trough, formalin treatment continued every eight hours until fry were hatching. If fungus became severe on an individual egg mass, the fungus was manually removed. When the fungus overwhelmed the eggs mass, caused all eggs to die, the entire egg mass was removed and discarded.

The eggs were allowed to develop within the paddle wheel troughs until an estimated 12 hours before hatch. At this time the percent of the embryos alive were determined and the egg mass weighed to allow calculation of percent hatch.

Incubation was the same in 2007 with the exception that the egg masses were moved midway through development from non heated water to heated water. Water temperature had dropped to 22.9˚C and was raised to 27.5˚C.

Data Analysis

Percentage ovulation was calculated as total number of fish that gave eggs divided by total number of fish implanted. Latency time was calculated using the number of hours from time of implantation to time of ovulation for only the fish that ovulated. Estimated percent hatch was calculated by determining the number of viable embryos at the time percent alive was recorded, an estimated 12 hours prior to hatch, divided by the total eggs in the original eggs mass. Relative fecundity, the number of eggs per kilogram female, was determined by number of eggs spawned divided by the ovulated female's body weight. Fry per kilogram female body weight equaled the total number of fry produced divided by kilograms of female, both ovulated and not ovulated.

Statistical analyses were conducted using Statistical Analysis System (SAS version 9.1 software). The select and control lines were compared within 2006 and 2007 spawning seasons. A t-test was conducted to compare means of variables at the 0.05 level of significance. A chi square was used to calculate any difference in the percent ovulation for the different lines. Realized heritability, $h^2 = R/S$; where R = response to selection and S = selection differential, were calculated for fecundity, hatch and fry/kg.

RESULTS

Three-Year-Old Females

The total number of gravid females for the 2006 season was 269 and the total number implanted was 251. The cull percent for 2006 was 6.7%. The mean relative fecundity for all females was 10,088 (Table 1). The mean latency for 2006 was 51.4 hours. The mean percent hatch was 32.5 and the average fry per kilogram female was 2,870 (Table 1).

The percent ovulation for the select progeny, 93.1, was not different from that of the control progeny, 87.0 (Table 1). There were no significant differences for individual traits between the select and control lines when they were three years old. The relative fecundity was 10,247 and 10,127 for the select and control lines, respectfully (Table 1). The average latency for select and control lines was 51.0 and 50.1 hours, respectfully (Table 1). The percent hatch and fry per kilogram female for select was 34.7 and 3,311 and for the control was 35.5 and 3,124 respectfully (Table 1). Realized heritabilities were 0.10, -0.03, and 0.06 for fecundity, percent hatch, and fry per kilogram female, respectively.

Four-Year-Old Females

The total number of gravid females for the 2007 season was 80 and the total number implanted was 63. The cull percent for 2007 was 21.3%. The average relative fecundity for all females during the 2007 spawning season was 8,957 (Table 1). The

average latency for all females was 59.8 hours for the 2007 spawning season. The average percent hatch for the 2007 spawning season was 11.0. The average fry per kilogram female for the 2007 season was 744 (Table 1).

The percent ovulation for the select progeny was 90.5 and for the control progeny was 85.7 (Table 1). The relative fecundity was 9,429 and 8,910 for the select and control lines, respectfully. The average latency for the select and control lines was 56.6 and 59.8 hours, respectfully. The percent hatch and fry per kilogram female for the select progeny was 9.8 and 835 and for the control progeny was 13.3 and 1,026, respectfully. Realized heritabilities were 0.42, -0.13, and -0.06 for fecundity, percent hatch and fry per kilogram female, respectively (Table 1).

DISCUSSION

The observed mean fry per kilogram for the select line was higher than that of the control when the fish were three years old, but the means were not statistically different. Realized heritability was 0.06. Although there was no statistical difference, an increase of 5.6 percent in a selection program could be economically significant in the long term. A 5.6 percent improvement, every generation, for 5 generations result in more than a 30 percent cumulative increase in fry per kilogram female.

The extreme variability found in the reproductive traits of ictalurid catfish (Hutson 2006), coupled with the existence of observations at zero, makes proof of statistical differences in reproductive evaluations in catfish difficult. Quintero et al. (2007a,b) examined several statistical models to address this problem and various evaluations can result in differences is statistical outcome. More research should address this problem.

The mean percent hatch for the select and control lines was nearly the same, so mass selection was not successful for this trait. Realized heritability was near zero, -0.03. The mean observed relative fecundity for the select line was higher than the control line, but again, there were no statistical differences found. The realized heritability was 0.10, in this case, which would be considered a low heritability.

Selection for fecundity, hatch rate and fry/kg did not affect the other reproductive traits, and no correlated responses to selection were observed. Cull percentage, percent ovulation, and latency period were not different for the select and control progeny.

When the channel catfish females reached four years of age, the observed mean fry per kilogram female and percent hatch for the select line were lower than the control line but no significant differences (P=0.05) were found. Realized heritability for percent hatch and fry per kilogram female were -0.06 and -0.13, respectively. The mean observed relative fecundity for the select line was higher than the control line, but again, there were no statistical differences found. Although not statistically significant, a 5.5 percent improvement was observed in the select line for fecundity in contrast to the 1.2 percent observed when they were three years old. In this case, realized heritability was 0.42, which would be considered high.

Again, at four years of age, selection for fecundity, hatch rate and fry/kg did not affect the other reproductive traits, and no correlated responses to selection were observed. Cull percentage, percent ovulation, and latency period were not different for the select and control progeny at four years of age.

The lower mean values in the reproductive traits such as percent hatch and fry per kilogram female, in the 2007 season, were likely complicated by environmental variables. These confounding factors included a lower number of degree days for the female brood fish, and low initial hatching temperature when a cold front arrived after spawning resulting in low hatch rates.

Based on the results from both years the heritability for hatch rate, when channel catfish females are induced to ovulate with LHRHa to produce hybrid embryos

artificially, is zero. No additive genetic variation was found for hatch rate. A potential economically significant improvement in fry output of the three year old females was observed, although heritability was extremely low. There was no improvement in fry output when the fish were four years old. This subtle difference could be a result of a genotype X environment interaction, with the severe cold conditions masking any genetic differences. Alternatively, genotype X age effect may explain the change in results. A response to selection for fecundity was observed for both years, with realized heritability ranging from 0.10-0.42. This trait appears to have additive genetic variation and can be improved via selection.

In general, the responses to selection and realized heritabilities attained in this study were lower than that found for other fish and shellfish. Acros (2004) reported that heritabilities for reproductive traits were low to zero, similar to the current results, but Macbeth (2007) reported high to low heritabilities for reproductive traits. Estimates for heritabilities of reproductive traits in fishes (Gall and Gross 1975, 1978, Gall et al. 1987, Gall and Huang 1988, Siitonnen and Gall 1989, Leary et al. 1989 Huang and Gall 1990, Sylven and Elvingson 1992, Crandell and Gall 1993, Ojanguren et al. 1996, Su et al. 1997) have usually been moderate to high. Moderate to high heritabilities were also estimated for rainbow trout reproductive traits egg number, egg volume, and egg size (Gall 1975, Gall and Gross 1978).

Additional factors may have influenced the responses to selection and realized heritabilities for the reproductive traits of channel catfish females in the current study. Selection differentials were quite high for fry per kilogram female and percent hatch. In this case, significant amounts of additive genetic variation can be difficult to identify

because heritability is a ratio, not an absolute value, and large amount of phenotypic variation due to environmental effects can mask genetic variation. This is perhaps further illustrated by the fact that the selection differential was much lower for fecundity, resulting in higher and positive heritabilities compared to percent hatch and fry per kilogram female. In this scenario, family selection can be more effective than mass selection.

Another explanation for the results might be that, inadvertently, the control line was also genetically improved, negating the measurement of significant selection responses. Maintaining an unselected control in this type of experiment is difficult because hatchling controls may have survived because of the same set of genes found in the select line. Fry per kilogram female and percent hatch for the progeny control was five to six times higher than that of the parental generation control, so performance of the control was dramatically improved. This could be due to improvements in husbandry, an inadvertent selection response or both. Further research should compare mass and family selection to improve the reproductive traits of channel catfish females and various designs should be evaluated to better measure performance of controls.

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