EVALUATION OF LHRHa IMPLANTS AND INJECTIONS ON THE PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE FRY

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Alison M. Hutson

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Date of Graduation

THESIS ABSTRACT

EVALUATION OF LHRHa IMPLANTS AND INJECTIONS ON THE PRODUCTION OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*Ictalurus furcatus*) MALE FRY

Alison M. Hutson

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The hybrid produced from the channel catfish (*Ictalurus punctatus*) female and the blue catfish (*I. furcatus)* male has better culture traits than the primarily cultured channel catfish. Producing hybrid catfish has been limited because of the low number of fry per kilogram produced. Increasing the number of fry per kilogram of female by using different LHRHa treatments to induce ovulation and increase sperm production were the goals of these experiments.

Two treatments, 100µg/kg implants and 30/150 injections, 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 and egg quality compared to females implanted with 100 µg/kg of LHRHa.

Latency time decreased with increasing temperatures. Females exposed directly or indirectly to males had higher fry per kilogram than females with no exposure. During 2005, females that were directly exposed to males, although consistent with the first year, had an increased latency of about 6 hours.

Higher doses of injections and implants, in general, tended to result in greater relative fecundity and greater numbers of eggs stripped from the females than lower doses of injections, and resulted in greater relative fecundity than lower doses of injections. Indirect contact and, therefore, probable pheromone communication increased hatch rate and fry/kg by about 3X.

In 2004, hatch of the first 4 egg masses stripped from an individual fish was essentially equivalent (20.7-23.2% hatch), and then hatch rate rapidly decreased for egg masses 5-7, 14.0, 7.6 and 6.5%, respectively. Effects of egg mass order were more dramatic in 2005. The first 3 egg masses had 28.6-31.2 % hatch, egg mass 4 had 12.2 % hatch and egg masses 5-6 4.1-4.4% hatch. The first 3-4 egg masses should give consistent and good hatch.

Strain of blue catfish had larger effects on reproduction than implantation. Large strain differences existed for sperm/g of testes, sperm/kg male body weight and hatching percentage.

Application of the best treatments examined in these experiments could result in commercial levels of hybrid fry production. Utilization of this hybrid would then have a positive impact on the economics of the American catfish industry.

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TABLE OF CONTENTS

LIST OF TABLES xi

I. INTRODUCTION 1

II. THE EFFECT OF THE USE OF LUTEINIZING HORMONE RELEASING HORMONE AGONIST IMPLANTS AND INJECTIONS ON OVULATION, LATENCY, FECUNDITY, EGG QUALITY, HATCH RATE, AND FRY PRODUCTION FOR CHANNEL CATFISH (*Ictalurus punctatus*) FEMALES HYBRIDIZED WITH BLUE CATFISH (*Ictalurus furcatus*) MALES 8

Abstract 9
Introduction 10
Materials and Methods 12
Results 22
Discussion 35
Literature Cited 38

III. THE EFFECT OF LUTEINIZING HORMONE RELEASING HORMONE AGONIST IMPLANTS ON SPERM PRODUCTION IN THE MALE BLUE CATFISH (*Ictalurus furcatus*)AND ON THE PRODUCTION OF THE CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE BLUE CATFISH MALE HYBRID FRY 39

Abstract 40
Introduction 41
Materials and Methods 44
Results 49
Discussion 51
Literature Cited 53

LIST OF TABLES

1 Treatment parameters for female channel catfish, *Ictalurus punctatus,* injected or implanted with luteinizing hormone releasing hormone analog and fertilized with blue catfish, *I.furcatus,* sperm in experiments 1, 2 and 3 in 2004. 17

2 Treatment parameters for female channel catfish, *Ictalurus punctatus,* injected or implanted with luteinizing hormone releasing hormone analog and fertilized with blue catfish, *I.furcatus,* sperm in experiments 4, 5, 6, 7 and 8 in 2005. 18

3 Percent ovulation, mean latency period, fecundity, fry per kilogram of female, hatching percentage, and egg quality of channel catfish females (*Ictalurus punctatus*) exposed or not exposed to channel catfish male 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 (*Ictalurus furcatus*) males (mean ± SD). 23

4 Percent ovulation, mean latency period, fecundity, fry per kilogram of female, percent hatch, and egg quality of channel catfish females (*Ictalurus punctatus*) by treatment 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 (*Ictalurus furcatus*) males (mean ± SD). 25

5 Effect of egg mass order on mean egg quality and percent hatch of channel catfish females (*Ictalurus punctatus*) 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 (*Ictalurus furcatus*) males (mean ± SD). 26

6 Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean + SD). Water temperature averaged 27.2 ºC. (N= no male exposure Y= exposure). 28

7 Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean + SD). Water temperature averaged 24.4 ºC, 25.1 ºC, and 24.9 ºC. (N= no male exposure Y= exposure). 29

8 Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean + SD). Water temperature averaged 27.8 ºC. (N= no male exposure Y= exposure). 30

9 Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean + SD). Water temperature averaged 27.2 ºC. (N= no male exposure Y= exposure). 32

10 Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD).Water temperature averaged 26.3 ºC and 28.0 ºC.(N= no male exposure Y= exposure). 33

11 The average number of sperm per gram of testes from the male, the average of sperm per kilogram of male BW, and percent hatch for the production of channel catfish female x blue catfish male hybrid fry. 50

12 Sperm/g of testes and sperm/kg of body weight for blue catfish, *Ictalurus furcatus,* males with and without 100ug/kg implants of GMP grade LHRHa, sacrificed 48 hours after implantation and mated with channel catfish,*I. punctatus* females. 51

INTRODUCTION

The channel catfish, *Ictalurus punctatus,* industry has become the largest aquaculture industry in the United States accounting for over 50% of all aquaculture production (Dunham et al 1993; Dunham et al 1998; Dunham et al 1999). More kilograms of channel catfish are produced per year (Tiersh et al 1990; Dunham et al 1993; Wolters and Johnson 1994; Dunham et al 1998; Liu et al 1998; Liu et al 2003; Rezk et al 2003). As aquaculture techniques become more developed, catfish production and production efficiency needs to be increased.

Catfish farming was initiated in the 1930s in Kansas and in the 1940s in Mississippi and in Arkansas, the production of channel catfish in hatcheries and farms has continued to grow, becoming an important culture species in the United States (Dunham and Smitherman 1984). The price of catfish received by producers from processors bottomed at 56.8 cents per pound in 2002 with the month of January being the lowest price at 52.9 cents. The 2006 price has rebounded to 75-80 cents per pound (The Catfish Farmers of America 2006), but historically, this is not a great price because of inflation, and farmers must prepare for future price fluctuations. Producers need to produce fingerlings that are resistant to disease, have a low feed conversion ratio, have a fast growth rate and are easily seined so that the catfish cost less to produce.

To increase performance for these and other commercially important traits, different methods to genetically enhance the channel catfish, *Ictalurus punctatus*, and blue catfish,

I. furcatus, are being evaluated. Growth has been improved (Dunham and Smitherman 1983; Dunham et al 1987; Dunham and Brummett 1999; Dunham et al 1999) via mass selection. Intraspecific crossbreeding has also improved performance in channel catfish (Dunham and Smitherman 1983, Padi 2003).

These breeding programs only improve one or a few traits at a time per strain. The interspecific hybrid, channel female X blue male, exhibits heterosis for many traits. The hybrid is a combination of the two most promising culture catfish species in the United States (Dunham et al 1993). Some culture traits that have been improved by producing a hybrid in comparison to the parent species include faster growth rate to market size (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al 1983; Dunham et al 1987; Dunham et al 1990; Dunham and Brummett 1999) and uniformity in growth rate for a cohort (Giudice 1966; Dunham et al 1982; Smitherman et al 1983; Argue et al 2003). Disease resistance has been improved in the hybrid (Dunham et al 1990). The hybrids also exhibit an increased tolerance to low dissolved oxygen (Dunham et al 1983) and uniformity in body shape (Dunham et al. 1982).

Hybrid catfish production has not been widely adopted in the industry because of the natural reproductive isolation between the channel catfish and the blue catfish that limits fry production (Tave and Smitherman 1982; Goudie et al 1993; Dunham et al 1998; Dunham et al 1999; Argue et al 2003; Kristanto 2004). Finding the appropriate spawning method to increase the percent fertilization of channel catfish eggs with blue catfish sperm will increase the production of the hybrids.

The channel blue hybrid is made by artificially spawning the channel and blue catfish. To prepare the fish for artificial spawning, many different hormones have been evaluated to stimulate ovulation and increase fry production. Using hormones such as human chorionic gonadotropin (hCG) and carp pituitary extract (CPE) have given some success for inducing ovulation in channel catfish females (Kim 1996). Using hormones to induce females to spawn can allow all of the females to spawn within the same time frame which would be beneficial for hatchery operations.

A hormone that is being studied for inducing ovulation in fish is luteinizing hormone-releasing hormone analog. LHRH induces the secretion of gonadotropins or luteinizing hormome (LH) (Zohar and Mylonas 2000). Without LHRH/GnRH, LH may still be produced, but will not release from the pituitary into circulation. Using injections of LHRHa releases LH, stimulating final oocyte maturation, ovulation and spermiation (Zohar and Mylonas 2000). One form of synthetic LHRH is produced by substituting the position 6 residue of native GnRH with a dextrorotary (D) amino acid and the position 10 amino acid with an ethylamide group, des-Gly10, [D-Ala6]LH-RH Ethylamide. LHRHa has been delivered as injections or implants.

Walleye (*Stizostedion vitreum*) have been spawned out of season by inducing ovulation with hCG and LHRHa with at least some of the hormone induced fish spawning 10, 6, and 3 weeks before the usual spawning season compared to the control group which had no early ovulation (Malison et al. 1998). Duncan et al. (2003) used LHRHa injections and implants to induce ovulation in bullseye puffer fish (*Sphoeroides annulatus*). The fish in the LHRHa injection and implant treatment groups had 82% ovulation compared to 18% ovulation in the control group receiving saline injections

(Duncan et al. 2003). The fertilization rates for the treated groups compared with the control were not significantly different, and both groups had a very wide range of fecundity. There was also no significant difference in percent fertilization between the LHRHa injection group, the LHRHa implant group, and the control. The injections and implants allowed the fish to have a more predictable spawning time which is necessary to artificial spawning but did not increase the productivity of the fish.

Using LHRHa injections or implants could increase the hatching percent and the number of fry produced per kilogram. If the appropriate dosage is identified commercial scale production of channel X blue hybrid fry may be possible. The objectives of this research were to identify the best dosages and method of delivery of LHRHa, determine latency periods for these treatments, compare efficiency of spawning environments and determine the effect of intraspecific pheromonal communication on the production of channel-blue hybrid catfish fry in channel catfish females. Additional objectives were to determine the effects of LHRHa implants on sperm production and sperm quality in blue catfish males for the production of channel-blue hybrid catfish fry.

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II. THE EFFECT OF THE USE OF LUTEINIZING HORMONE RELEASING HORMONE ANALOGUE IMPLANTS AND INJECTIONS ON OVULATION, LATENCY, FECUNDITY, EGG QUALITY, HATCH RATE, AND FRY PRODUCTION FOR CHANNEL CATFISH (*Ictalurus punctatus*) FEMALES HYBRIDIZED WITH BLUE CATFISH (*Ictalurus furcatus*) MALES

ABSTRACT

LHRHa injections were given in two doses, a primary dose and a resolving dose. 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 X blue catfish male hybrid embryos.

Two treatments, 100µg/kg implants and 30/150µg/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 and egg quality compared to females implanted with 100 µg/kg of LHRHa.

In 2004, hatch of the first 4 egg masses stripped from an individual fish was the highest (20.7-23.2% hatch), and then hatch rate rapidly decreased for egg masses 5-7, 14.0, 7.6 and 6.5%, respectively. Effects of egg mass order were more dramatic in 2005. The first 3 egg masses had 28.6-31.2 % hatch, egg mass 4 had 12.2 % hatch and egg masses 5-6 4.1-4.4% hatch.

In 2004 and 2005, channel catfish females implanted with LHRHa produced the most fry per kilogram of fish. Latency time decreased with increasing temperatures. Females exposed directly or indirectly to males had higher fry per kilogram than females with no exposure. During 2005, females that were directly exposed to males, although consistent with the first year, had an increased latency of about 6 hours.

INTRODUCTION

Luteinizing hormone releasing hormone analogue or mammalian gonadotropin hormone releasing hormone was discovered in the hypothalamus of mammals in the 1970's (Schally 1978). LHRH induces the secretion of gonadotropins or luteinizing hormome (LH) (Zohar and Mylonas 2000). Without LHRH/GnRH, LH may still be produced, but will not release from the pituitary into circulation. Bruzska and Adamek found that in artificial spawning of European catfish, *Silurus gianis L.*, LHRH analog, LHRHa given at 20µg/kilogram of female stimulated 100% ovulation. Forty-eight hours after fertilization, the LHRHa group and the group stimulated with Ovaprim, a compound containing hormone and the dopamine antagonist, had the best egg quality (Bruzska and Adamek 1999).

LHRHa has shown potential for inducing ovulation for artificial spawning in the production of the hybrid channel catfish, *Ictalurus punctatus,* female X blue catfish, *I. furcatus,* male (Kristanto 2004). Different doses need to be evaluated to find the appropriate dose for induced ovulation at different times in the spawning season. The channel catfish females that are spawned in May at the beginning of the spawning season have been exposed to a minimum number of degree days compared to later in the season. As the number of degree days increases late in the spawning season, the strength of hormone used may need to be decreased to be effective and prevent over ripening of the eggs (Kristanto 2004)

Large-scale commercial adoption of the hybrid between female channel catfish and male blue catfish would revolutionize the catfish industry which today is almost entirely based on the culture of channel catfish. Behavioral and reproductive isolating mechanisms between the two species prevent consistent commercial scale production of the hybrid. Induced spawning and artificial fertilization is an option to overcome this barrier.

Previously luteinizing hormone releasing hormone analogue, LHRHa, injections and implants were demonstrated to be more effective than carp pituitary extract, gonadotropin hormone releasing hormone (GnRH), salmon GnRH, and ovaprim, a synthetic analogue of a gonadotropin releasing agent and dopamine inhibitor, injections for producing channel catfish female X blue catfish hybrid embryos (Kristanto 2004). Ovaprim contains an analog of salmon gonadotropin releasing hormone (GnRH) and a dopamine inhibitor (domperidone) that is required for successful artificial spawning in many cultured fish species. It has been applied in several fish species (Syndel, 2001).

The objective of this experiment was to evaluate different injection and implant doses of LHRHa to determine the best dose throughout the spawning season to increase egg quality, egg quantity and fry/kg female body weight. The overall objective is to increase the efficiency of channel-blue hybrid catfish fry production.

MATERIALS AND METHODS

All experimental fish were kept in earthen ponds throughout the year at the North Auburn Fisheries Unit. The different strains of fish were marked with a heat brand or a pit tag for identification. The males and females were seined and transported to tanks to be prepared for spawning. Males and females were selected by strain and by the visible reproductive readiness such as indicated by head size for the males and abdominal distention for the females.

The females for spawning were held in tanks measuring 3.0 x 0.47 x 0.61 m and with a water volume from 670 – 837 liters. Each tank had a constant flow through and compressed air for aeration.

The male blue catfish were brought into the laboratory and sacrificed to obtain sperm. The sperm was prepared approximately 24 hours before the females began to ovulate and then placed in the refrigerator.

Injections and Implants

To induce ovulation, the females received two injections of liquid LHRHa or a single LHRHa implant. One milligram of LHRHa was mixed with 0.85% saline solution to make a 1,000 µg/ml stock solution. The females were given a priming dose followed by a resolving dose twelve hours later. The injections were given intraperitoneally. The fish were kept in one of three spawning environments, bags inside a tank, loose in the tank, or in aquaria. The fish in the bags were weighed before being put in the bags. The

bags were labeled with the fish's weight and hung on the side of the tank with clothespins. The fish were then injected through the bag. The fish held loose in the tanks had to be weighed before both injections to deliver the appropriate dose.

The implants were given in a single dose just behind the dorsal fin and ventrally down the body approximately 3cm. The implanted fish were kept in the same spawning environments as the injected fish, in bags, left loose in the tanks, or held in an aquarium.

Sperm Preparation

The males were weighed, sacrificed and then their testes were removed. The testes were cleaned with saline solution and trimmed with scissors to remove excess tissue and blood. The testes were weighed and placed in a clean plastic freezer bag. Then up to 10 ml of saline was added. The testes were placed in a clean plastic freezer and were mashed in the bag together with half of the total saline. The contents of the bag were then rinsed with the leftover amount of saline to bring the final solution to10 ml of salt solution per gram of testes.

Artificial spawning

Ovulation occurred approximately 40 to 55 hours after the priming dose of the injections and 45 to 60 hours after implants. All females were checked for eggs every 3 hours after the first fish gave eggs. The females were checked for eggs by gently pushing on the abdomen and rubbing gently from head to tail. If the females were not ovulating they were returned to their tank and checked again. Bags were checked for eggs visually or by gently lifting and moving the bag to minimize the disturbance of the female. If eggs were present on the bag, the fish and the bag were removed from the water and placed in the anesthesia. The fish held in aquaria were allowed to initiate egg deposition

in the tank and were then removed to hand strip. The fish held in aquaria without males were left in the aquaria until they began to ovulate. They were monitored in the tank until ovulation. When the females were giving eggs freely, they were placed in a solution with 200 ppm MS 222 and 200 ppm sodium bicarbonate until their movement slowed. The fish were then dipped into a tank of freshwater while the vent was covered by a finger to keep the eggs from leaking out while rinsing off the anesthesia. The fish was placed on a dry towel and the head was covered with a towel to catch any water leaking from the gill cavity. The fish were then taken to the stripping table and hand stripped. The eggs were stripped into pie pans greased with a thin layer of vegetable shortening to prevent sticking. The females were stripped of eggs until the eggs no longer flowed. The pie pans generally were filled with 70-150g of eggs and then a new pan was started so that all females had several egg masses.

Fertilization

The eggs were fertilized within minutes of the eggs being stripped and weighed. The eggs were rinsed with saline solution to remove all the blood and excess tissue from the female. If no blood was present, the eggs were not rinsed. The sperm solution was drawn into a 1 ml or 3 ml syringe. In 2004, the amount of sperm was based on the number of grams of sperm per milliliter of salt solution. For every gram of testes, ten milliliters of saline solution were used. The eggs were fertilized with 2.5 milliliters of sperm/salt solution per 100 grams of eggs. In 2005, 6.5×10^7 sperm per 100g of eggs were used for fertilization. The amount of sperm per milliliter of solution was determined by diluting the sperm sample 10 to 50 times and then determining absorbance of the sample with a spectrophotometer at 546 nanometers. A graph was constructed

using regression analysis by counting the number of sperm in the undiluted solution and then running the sample through the spectrophotometer to find the frequency determining the sperm number in the serial dilutions with the spectrophotometer. The numbers were then plotted so that the number of sperm could be calculated from the spectrophotometer reading.

The sperm was added to the egg mass in a circular motion to expose all the eggs to the sperm solution. Dechlorinated water was then added to the egg mass and sperm solution in the pie pan to activate the eggs and begin the fertilization process. The egg and sperm were gently swirled together. The fertilized egg masses were then allowed to sit for 2 to 10 minutes until they formed a mass and were then transferred to a water hardening trough. The eggs remained in the water hardening trough for at least 15 minutes. In the water hardening trough there was constant water flow and aeration. The eggs were transferred to an egg basket in a paddle wheel hatching trough. The troughs had an air supply and a paddle wheel which was turned on when the youngest egg mass in the trough was at least 3 hours old.

Incubation

The eggs were held in tanks with paddlewheels until hatch. The eggs treatment began 12 hours after they were fertilized. The initial treatment was always formalin (100ppm) and then the eggs were treated three times a day, the first with copper sulfate (32ppm), the second with formalin, and the last with copper sulfate to help prevent fungus growth until they began to hatch. The eggs were not treated between 42 to 46 hours at 28ºC after fertilization during 2005 because that was found to be a critical period of development adversely affected by formalin treatments (Small and Chatakondi, 2006).

Experiments 2004

Experiments 1, 2, and 3 were conducted in 2004. In experiment 1, the females were exposed to different LHRHa treatments and different spawning environments, in aquaria stocked or not stocked with conspecific males, held loose in tanks, or held in bags (Table 1). The average temperature was 27.2º C.

In experiment 2, the females were divided into seven treatment groups with the injection or implant dose and the spawning environment varying (Table 1.). The water temperature averaged 27.5 º C º.

For experiment 3 all females were held in bags (Table 1.). The temperature averaged 28 º C.

Experiments 2005

Experiments 4, 5, 6, 7 and 8 took place during the 2005 spawning season. In experiment 4, all females were given an injection with a priming dose of 30 µg/kilogram of female and a resolving dose of 150µg/kg (Table 2). For injections the injected powder was 82% active ingredient, so actual dose, for instance, was 82µg/kg for fish injected with 100 µg/kg of the powder. All fish were held in bags inside tanks after they were injected until ovulation. The average temperature was 24.4 º C.

For experiment five, fifty one females were implanted with a time release LHRHa implant. The fish were placed into aquaria (Table 2). Each individual aquarium was divided into two by placing a plastic separator into the tank. Some females had males in the aquarium and others had no male exposure. The fish were allowed to display spawning behavior with the males, and the males were permitted to begin spawning.

Table 1. Treatment parameters for female channel catfish, *Ictalurus punctatus,* injected or implanted with luteinizing hormone releasing hormone analog and fertilized with blue catfish, *I.furcatus,* sperm in experiments 1, 2 and 3 in 2004.

Experiment	Treatment group	N	Priming dose (µg/kg)	Resolving dose (µg/kg)	Implant dose (Single Implant) (µg/kg)	Environment	Male Exposure
1	1	10	10	50	-------	Loose	No
	2	10	20	100	-------	Bag	No
	3	10	20	100	-------	Loose	No
	4	10	30	150	-------	Loose	No
	5	10	-------	-------	75	Loose	No
	6	7	-------	-------	100	Aquaria	No
	7	8	-------	-------	100	Aquaria	Yes
	8	10	-------	-------	125	Loose	No
2	1	7	10	50	-------	Bag	No
	2	7	20	100	-------	Bag	No
	3	7	20	100	-------	Loose	No
	4	6	30	150	-------	Bag	No
	5	7	-------	-------	75	Bag	No
	6	8	-------	-------	75	Loose	No
	7	8	-------	-------	100	Bag	No
3	1	7	10	50	-------	Bag	No
	2	8	10	150	-------	Bag	No
	3	7	20	100	-------	Bag	No
	4	8	20	100	-------	Bag	No
	5	7	30	150	-------	Bag	No
	6	8	-------	-------	75	Bag	No
	7	8	-------	-------	75	Bag	No
	8	8	-------	-------	100	Bag	No
	9	8	-------	-------	100	Bag	No

Table 2. Treatment parameters for female channel catfish, *Ictalurus punctatus,* injected or implanted with luteinizing hormone releasing hormone analog and fertilized with blue catfish, *I. furcatus,* sperm in experiments 4, 5, 6, 7 and 8 in 2005.

Experiment	Treatment group	N	Priming dose (µg/kg)	Resolving dose (µg/kg)	Implant dose (Single Implant) (µg/kg)	Environment	Male Exposure
4	1	34	30	150	-------	Bag	No
5	1	32	------	-------	100	Aquaria	Yes Direct
	2	10	------	-------	100	Aquaria	Yes Indirect
	3	5	------	-------	100	Aquaria	No
6	1	10	------	-------	50	Bag	No
	2	10	------	-------	100	Bag	No
	3	10	150	-------	-------	Bag	No
7	1	10	-------	-------	75	Bag	No
	2	10	-------	-------	100	Bag	No
8	1	23	-------	-------	75	Bag	No
	1	23	-------	-------	100	Bag	No

The females were then removed from the aquaria and then strip spawned. The temperature averaged 25.1 º C.

Experiment six was the first experiment using varying doses of LHRHa of the 2005 spawning season. All of the females were held 10 fish to a tank in bags. There were three treatment groups, two implant and one injection. The implanted fish were given either a 50µg/kilogram of fish implant or a 100µg/kg implant. The injected fish received a single injection instead of a priming dose and a resolving dose as done in the previous experiments. The injection was accomplished with 150 µg/kg of liquid LHRHa (Table 2). The temperature averaged 24.9 º C.

For experiment 7, 20 fish were divided into two treatment groups. The females were given a 75 or 100µg/kg implant. All of the females were held in bags (Table 2). Two treatment groups of males were used, one receiving an implant and the other a control group with no implant. The males were implanted with LHRHa into the muscle tissue just behind the dorsal fin. The males were given a 48 hour latency period prior to sacrifice for testes removal. The testes were removed in the same manner as above but some males were sacrificed and surgery was done on the remaining males. The amount of sperm in the testes from each individual fish was calculated from a spectrophotometric reading. The average temperature was 26.3 º C.

Experiment 8 used the same treatment groups as in experiment 7 (Table 2.) The final spawn of the season occurred late in the spawning season so the fish had been exposed to the maximum number of degree days. The water temperature averaged 28.0 º C.

Data Analysis

The treatments were different hormone concentrations and delivery method, exposure or non-exposure of the female to conspecific males and blue catfish males with or without LHRHa implant in various experiments. Percentage ovulation was calculated as number of fish giving eggs divided by total number fish for each treatment. Latency time was calculated using the number of hours from the first injection or implant to time of ovulation. The average latency was the average latency by treatment of only the fish that ovulated. Hatch was calculated by determining the number of viable embryos 12 hours prior to hatch divided by the total eggs in the original egg mass. The number of eggs per kg female body weight (relative fecundity) was determined by number of eggs spawned divided by female body weight for females that ovulated. Fry per kilogram equaled the total number of fry produced divided by the weight of the total number of females in the treatment. Egg quality was determined on a scale from 1 to 5. The score of 5 was assigned for good quality free flowing eggs with yellow color and without blood, a score of 4 indicated free flowing eggs that were sticky and with a pale yellow color, a score of 3 was assigned for free flowing eggs with clumps and blood present, a score of 2 was given to free flowing eggs containing clumps, blood and extra fluid, and a score of 1 was assigned for white eggs with excessive blood, clumps and extra fluid. Egg quality was assigned for each egg mass in the order they were stripped from the fish. The egg masses consisted of approximately 150g of eggs.

As a part of egg quality, the egg masses were also examined for good eggs, blood, fluid, clumps, or white eggs within the egg mass. The different conditions were either absent or present and the percentage of eggs with egg quality was calculated.

Experiments four and five do not have different LHRHa concentrations. The single treatments were compared to the other experiments during the 2005 spawning, and egg quality and hatching percentage were compared among egg strippings within an experiment. Statistical analysis of data and other was conducted using Statistical Analysis System 9.1 (SAS Institute 2004). Analysis of Variance was used to determine if treatments had significant effects. Duncan's Multiple Range Tests (DMRT, Duncan 1955) was conducted to compare means of variables that showed significant responses at the 0.05 level of significance. A chi square test was calculated to find any difference in the percent ovulation for the different treatments.

RESULTS

Male Exposure Effects by Season

During season one, ovulation rates for females that were directly exposed to males or not exposed to males were different ($P<0.05$). Females exposed to males had a higher ovulation rate. The same result was obtained for 2005. There was a difference in latency time for the fish that were exposed to males compared to the fish that were not exposed to males ($P<0.05$) (Table 3). Direct exposure to males delayed ovulation by 6 hours. However, during 2005, there was not ($P>0.05$) a significant latency difference among the females that were not exposed to males, the females that were indirectly exposed to males, and the females that were directly exposed to males, although consistent with the first year, direct exposure to males increased latency by about 6 hours.

The effect of no male exposure was observed for fecundity for either year. During year 2005, there was a hatch rate varied among all three treatment groups ($P<0.05$). Indirect exposure to males, direct exposure to males and no exposure had mean hatch rates of 62.0, 41.5 and 19.9%, respectively. There was also a difference in egg quality during 2005 that was not seen in 2004 ($P<0.05$). The females exposed directly to males had the highest egg quality in both years although the only difference was in 2005 ($P<0.05$). Despite having the highest ($P<0.05$) egg quality, these females had intermediate hatch rate. No differences ($P>0.05$) in fry/kg were found, although observed means were higher for exposed females and were consistent with significant differences in hatch rate.

Table 3. Percent ovulation, mean latency period, fecundity, fry per kilogram of female, hatching percentage, and egg quality of channel catfish females (*Ictalurus punctatus*) exposed or not exposed to channel catfish male 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 (*I. furcatus*) males (mean ± SD).

Year	Male Exposure	N (Females	Mean Percent Ovulation*	Latency	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Percent Hatch	Egg Quality
2004	N	170	62.4	47.2^{b} ± 6.8	9,131^{a} ± 2,906	534 ± 1289	170	20.8 ± 31.2	3.8^{a} ± 1.0
	YD	8	62.5	53.1^{a} ± 2.7	10,059^{a} ± 3,140	1449 ± 0	8	12.7 ± 0.0	4.0^{a} ± 0.8
2005	N	165	62.2	62.6^{a} ± 14.5	10,967^{a} ± 3,044	1,350 ± 1,660	398	19.9^{c} ± 27.8	3.7^{b} ± 1.0
	YI	10	50.0	62.8^{a} ± 9.7	11,944^{a} ± 3,660	4,322 ± 853	25	62.0^{a} ± 31.3	3.8^{b} ± 0.9
	YD	32	78.1	69.0^{a} ± 10.5	10,676^{a} ± 3,040	2,188 ± 2,440	97	41.5^{b} ± 42.4	4.3^{a} ± 0.8

a,b,c means followed by the same letter are not different (P>0.05, Duncan's Multiple Range Test) within each column

* Percent ovulation is significantly different (P=0.05, Pearson's chi-square test)

Treatment Effects by Year

Ovulation rates were different in three treatment groups, 20/100 (priming dose µg/kg /resolving dose µg/kg), 30/150, and 100 (single dose (µg/kg)) implant (P<0.05). Latency period was different (P<0.05) by treatment during season one and season two (Table 4). Due to warmer temperatures in 2004, the latency period was shorter for almost every treatment. The treatment group receiving a priming dose of 10µg/kg of female and a resolving dose of 150µg/kg had only a 25% ovulation percent, but had the shortest (P<0.05) latency period of all treatment groups. During 2004, fecundity was highest (P<0.05) for the 100 and 125 implant treatment groups. The two groups were different from the 10/50 and 20/100 injection groups. There were no differences (P>0.05) in fecundity between treatment groups during 2005. Fry per kilogram of female was not different (P>0.05) between treatment groups for the 2004 and 2005 spawns. There were differences in hatch for both seasons (P<0.05). During season one, the 20/100 treatment group had the highest hatch. During season two, the 100 implant group had the highest (P<0.05) hatch when the groups were compared by season. Egg quality for the 30/150 injection and the 75 implant treatments during 2005 were higher (P<0.05) than the 150/0 injection group. No egg quality differences were observed in 2004.

Egg Mass Order

The egg mass order from the fish affected (P<0.05) both egg quality and percent hatch (Table 5). The first three egg masses that were stripped from an individual female had the highest egg quality and the highest percent hatch. The correlation (P=0.001) between hatch rate and egg mass order was -0.95.

Table 4. Percent ovulation, mean latency period, fecundity, fry per kilogram of female, percent hatch, and egg quality of channel catfish females (*Ictalurus punctatus*) by treatment 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 (*I. furcatus*) males (mean ± SD).

Year	Treatment	N (Females)	Mean Percent Ovulation*	Latency	Fecundity	Fry per Kilogram of Female	N (Egg Masses)	Percent Hatch	Egg Quality
2004	10/50	24	75	51.1^{b} ± 5.8	8,022^{b} ± 4,021	464 ± 880	63	23.9^{a,b,c} ± 30.2	3.3^{a} ± 1.4
	10/150	8	25	42.5^{d} ± 2.1	NA	0.0 ± 0.0	10	0.0^{c} ± 0.0	4.0^{a} ± 1.0
	20/100	41	61	46.1^{c} ± 5.1	7,914^{b} ± 2,866	599 ± 1,157	97	39.6^{a} ± 41.4	3.9^{a} ± 0.8
	30/150	24	58.3	45.6^{c} ± 4.2	9,529^{a,b} ± 2,180	576 ± 1,390	57	31.1^{a,b} ± 34.9	4.1^{a} ± 0.8
	75	37	56.8	47.8^{c} ± 7.9	9,731^{a,b} ± 2,645	212 ± 488	85	7.1^{b,c} ± 13.9	4.1^{a} ± 0.8
	100	34	61.8	45.4^{c} ± 7.8	10,844^{a} ± 2,313	1,065 ± 2,095	92	14.4^{a,b,c} ± 25.5	3.8^{a} ± 1.1
	125	10	100	56.7^{a} ± 1.5	10,353^{a} ± 1,402	754 ± 0	34	5.7 ± 0.0	3.6^{a} ± 1.0
2005	30/150	66	65.2	56.7^{c} ± 7.6	10,203^{a} ± 2,319	1,295 ± 1,714	130	20.2^{a,b} ± 25.9	4.0^{a} ± 0.9
	150/0	10	60	69.2^{b} ± 12.0	10,178^{a} ± 5,829	540 ± 862	22	19.0^{a,b} ± 36,8	3.4^{b} ± 1.1
	50	10	100	83.2^{a} ± 14.1	12,452^{a} ± 2,255	1,326 ± 1,359	24	19.1^{a,b} ± 27.7	3.5^{a,b} ± 0.8
	75	26	53.9	64.4^{b,c} ± 14.3	NA	675 ± 587	71	8.7^{b} ± 11.8	3.9^{a} ± 0.9
	100	101	58.4	64.7^{b,c} ± 14.0	11,453^{a} ± 3,233	2,278 ± 2,199	270	34.0^{a} ± 37.6	3.8^{a,b} ± 1.0

a,b,c means followed by the same letter are not different (P>0.05, Duncan's Multiple Range Test) within each column
* Percent ovulation is significantly different (P=0.05, Pearson's chi-square test)

Table 5. Effect of egg mass order on mean egg quality and percent hatch of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD).

Year	N (Egg masses)	Egg Mass Order	Mean Egg Quality	Percent Hatch*
2004	113	1	4.0 ± 1.0	23.2a ± 34.4
	105	2	3.9a ± 0.9	22.1a ± 32.2
	78	3	3.8a ± 0.9	20.7a ± 34.4
	49	4	3.6a ± 1.0	21.7a ± 28.3
	18	5	3.0a,b ± 0.7	14.0 ± 19.2
	6	6	2.5b ± 1.2	7.6a ± 11.8
	2	7	4.0a ± 1.4	6.5a ±9.2
2005	159	1	4.1a ± 0.9	31.2a ± 36.6
	88	2	3.8a ± 1.0	28.6a ± 32.9
	122	3	3.7a ± 0.9	28.6a ± 32.5
	36	4	3.4a ± 0.9	12.2a,b ± 24.9
	19	5	2.7b ± 0.5	4.1b ± 6.9
	10	6	NA	4.4b ± 9.5

a,b,c means followed by the same letter are not different (P>0.05, Duncan's Multiple Range Test) within each column

* The correlation (P=0.001) between hatch rate and egg mass order was -0.95.

Treatment Effects by Experiment

Experiment 1

During experiment one, two treatments, 20/100 bag and 125 implant, had 100 percent ovulation (Table 6). The females held in aquaria without males given a 100µg/kg. implant had the lowest ovulation percentage of 57.1 ($P<0.05$). There were differences ($P<0.05$) in latency time, egg quality and fecundity among treatments ($P< 0.05$). Average latency time ranged from 47.7 hours for 20/100 bag to 58.5 hours 100 implant. Egg quality was above 3.5 on a 5 point scale for all treatments. Total fry rather than fry per replicate were available so statistical analysis was not accomplished. The numbers ranged from 0 fry produced for the 10/50 µg/kg injection treatment when the fish were held loose in a tank with no male exposure to 1,538 fry per kilogram for the 100 µg/kg implant treatment with the females held in aquaria and exposed to males.

Experiment 2

There was no difference between treatments for ovulation percentage ($P> 0.05$). Ovulation ranged from 28.6 percent to 80.0 percent (Table 7). Differences in latency time, egg quality, fecundity and fry per kilogram of female were observed among treatment groups ($P< 0.05$).

Experiment 3

Experiment three had the lowest observed overall ovulation percentage (Table 8). No differences were observed for ovulation percentage and egg quality among treatments

Table 6. Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD). Water temperature averaged 27.2 ºC. (N= no male exposure Y= exposure)

Experiment	Treatment, Male Exposure, Spawning Environment	N (Females)	Delivery Method	Mean Percent Ovulation*	Latency Time	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
1	10/50 N Loose	10	Injection	80.0	56.2[a] ± 3.4	6,711[b] ± 4,279	0	29	3.5[c] ± 1.3	0
	20/100 N Loose	10	Injection	66.7	52.1[b,c] ± 5.0	6,756[b] ± 2,996	1389	23	4.4[a] ± 0.9	21.8
	20/100 N Bag	9	Injection	100.0	47.7[d] ± 5.2	9,670[a,b] ± 2,052	843	25	4.0[a,b] ± 0.4	12.7
	30/150 N Loose	10	Injection	90.0	52.2[b,c] ± 0.3	9,447[a,b] ± 2,482	800	31	4.3[a] ± 0.6	9.4
	75 N Loose	10	Implant	80.0	49.3[c,d] ± 3.8	8,874[a,b] ± 4,894	455	28	4.1[a,b] ± 0.8	5
	100 N Aquaria	8	Implant	57.1	58.5[a] ± 2.3	11,833[a] ± 1,762	1449	20	4.1[a,b] ± 0.8	19.5
	100 Y Aquaria	7	Implant	62.5	53.1[b] ± 2.7	10,059[a,b] ± 3,140	1538	19	4.0[a,b,c] ± 0.8	21.9
	125 N Loose	10	Implant	100.0	56.7[a] ± 1.5	10,353[a,b] ± 1,402	734	34	3.6[b,c] ± 1.0	5.7

a,b,c means followed by the same letter are not different (P>0.05, Duncan's Multiple Range Test) within each column

* Percent ovulation is significantly different (P=0.05, Pearson's chi-square test

Table 7. Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD). Water temperature averaged 27.5 ºC.

Experiment	Treatment, Male Exposure, Spawning Environment (N=no male exposure)	N (Females)	Delivery Method	Mean Percent Ovulation	Latency Time	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
2	10/50 N Bag	7	Injection	71.4	44.6^{a,b} ± 2.0	5,310 ± 6,027	862^{b} ± 1,072	21	2.5^{c} ± 1.2	29.9^{a,b} ± 32.4
	20/100 N Loose	7	Injection	28.6	45.8^{a} ± 0.3	2,141 ± 3,785	393^{b} ± 1,040	13	3.3^{b} ± 0.5	29.1^{a,b} ± 30.1
	20/100 N Bag	7	Injection	71.4	40.9^{b} ± 4.3	5921 ± 4,802	1,341^{b} ± 1,663	26	3.4^{b} ± 0.7	49.7^{a} ± 43.7
	30/150 N Bag	7	Injection	43.0	43.5^{a,b} ± 3.5	4,189 ± 5,267	1,120^{b} ± 1,941	16	3.9^{a,b} ± 0.9	44.1^{a} ± 33.9
	75 N Bag	5	Implant	40.0	45.2^{a} ± 2.6	3,453 ± 4,843	634^{b} ± 887	10	3.7^{a,b} ± 0.8	28.0^{a,b} ± 18.5
	75 N Loose	7	Implant	42.9	44.7^{a,b} ± 4.3	5,126 ± 4,223	230^{b} ± 367	15	4.4^{a} ± 0.8	9.7^{b} ± 12.9
	100 N Bag	5	Implant	80.0	42.8^{a,b} ± 3.0	8,669 ± 5,059	3,394^{a} ± 3,316	15	3.9^{a,b} ± 0.9	39.4^{a,b} ± 29.6

a,b,c means followed by the same letter are not different (P>0.05) within each column

Table 8. Percent ovulation, mean latency period, hatching percentage, fecundity fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD). Water temperature averaged 28.0ºC.

Experiment	Treatment, Male Exposure, Spawning Environment (N=no male exposure)	N(Females)	Delivery Method	Mean Percent Ovulation	Latency Time	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
3	10/50 N Bag	7	Injection	71.4	50.3[a] ± 2.4	9,647[a] ± 2,724	0	13	4.5 ± 0.8	13.5 ± 23.9
	10/150 N Bag	8	Injection	25	42.5[b,c] ± 2.1	1,449[b] ± 2,721	0	10	4.0 ± 1.0	0
	20/100 N Bag	8	Injection	25	42.1[b,c] ± 1.7	1,808[b] ± 3,423	0	10	3.5 ± 1.0	0
	30/150 N Bag	7	Injection	29	43.0[b] ± 1.2	1,610[b] ± 2,849	0	10	3.4 ± 0.9	0
	75 N Bag	15	Implant	53	40.8[b,c] ± 2.9	7,210[a,b] ± 9,066	10 ± 33	32	4.0 ± 0.8	0.9 ± 0.5
	100 N Bag	14	Implant	57.1	39.9[c] ± 2.9	6,431[a,b] ± 7,846	173 ± 632	38	3.6 ± 1.3	3.5 ± 13.0

a,b,c means followed by the same letter are not different (P>0.05) within each column

(P> 0.05). Egg quality ranged from 3.4 to 4.5 but there were large standard deviations across treatments. Differences for latency time and fecundity were observed. Percent hatch was near zero for experiment three as was fry per kilogram of female likely due to poor quality sperm.

Experiment 5

Ovulation rate was different among treatments (P< 0.05) with females indirectly exposed to males having the lowest observed mean (Table 9). Ovulation rate ranged from 50 to 80%. No significant differences in treatment were observed for any of the variables measured (P> 0.05), although the observed mean for fry/kg was twice as high, 6,649, for indirectly exposed females compared to directly exposed females, 3,232.

Experiment 6

The 50 µg/kg implant group had a longer (P< 0.05), 83.2 hr, latency time than the 150 µg/kg injection treatment, 69.2 hr (Table 9). No difference in fecundity or hatch were observed (P>0.05). A difference fry per kilogram of female was seen (P< 0.05). Means for 100µg implant treatment, 50µg implant and 150 injection were 3,846, 1,500 and 831 fry/kg, respectively

Experiment 7

Ovulation rates varied from 60% for the 75 µg/kg implant to 83% for the 100 µg/kg implant (Table 10) but were not different (P>0.05). Average latency time ranged from 40.5 to 44.5 hours for the two groups. The 100 µg/kg implant was the best observed dose of the two producing ovulation in females at 40.5 hours with a standard deviation of 2.2 with over 83% ovulation. No difference in percent hatch between the two treatments of 75 µg/kg implant and the 100 µg/kg implant was observed (P> 0.05).

Table 9. Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD). Water temperature averaged 24.4 ºC, 25.1 ºC, and 24.9 ºC.

Experiment	Treatment, Male Exposure, Spawning Environment (N=no male exposure)	N (Females)	Delivery Method	Mean Percent Ovulation	Latency Time	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
4	30/150 N Bag	66	Injection	65	56.7 ± 7.6	10,203 ± 2319	2,072 ± 2,742	130	4.0 ± 0.9	20.2 ± 25.9
5										
	100 N Aquaria	5	Implant	80	67.3 ± 8.6	13,793 ± 2,489	5,321 ± 4,845	22	NA	46.1 ± 28.7
	100 YD Aquaria	32	Implant	78.1	69.0 ± 10.5	10,676 ± 3,040	3,232 ± 3,736	101	4.3 ± 0.8	41.5 ± 42.3
	100 YI Aquaria	10	Implant	50	62.8 ± 9.7	11,944 ± 3,660	6,649 ± 1,313	25	3.8 ± 0.8	62.0 ± 31.3
6	50 N Bag	10	Implant	100	83.2[a] ± 14.1	12,452 ± 2,255	1,500[b] ± 1,926	34	3.6 ± 0.8	19.1[a] ± 27.7
	100 N Bag	10	Implant	90	78.0[a,b] ± 8.8	12,198 ± 3,718	3,846[a] ± 2,146	34	3.7 ± 0.8	34.3[a] ± 35.1
	150 N Bag	10	Injection	60	69.2[b] ± 12.0	10,178 ± 5,829	831[b] ± 1,327	22	3.4 ± 1.1	19.0[a] ± 36.8

a,b,c means followed by the same letter are not different (P>0.05) within each column
* indicates a significant difference.

Table 10. Percent ovulation, mean latency period, hatching percentage, fecundity, fry per kilogram of female, and egg quality of channel catfish females (*Ictalurus punctatus*) 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 (*I. furcatus*) males (mean ± SD). Water temperature averaged 26.3 ºC and 28.0 ºC.

Experiment	Treatment (N= no male exposure)	N (Females)	Delivery Method	Mean Percent Ovulation	Latency Time	Fecundity	Fry per Kilogram of Female	N (Egg masses)	Egg Quality	Percent Hatch
7	75 N Bag	10	Implant	60	44.5 ± 8.9	8,030 ± 7,383	623 ± 861	40	NA	8.7[a] ± 11.8
	100 N Bag	8	Implant	83.3	40.5 ± 2.2	13,379 ± 3,153	1,020 ± 978	49	4.7 ± 0.6	9.5[a] ± 20.3
8	75 N Bag	8	Implant	100	70.0 ± 9.6	10,651 ± 1,869	0	34	3.9[a] ± 0.9	0
	100 N Bag	9	Implant	25.9	62.2 ± 10.1	5,996 ± 5,103	0	34	3.0[b] ± 1.2	0

Experiment 8

A significant difference in ovulation rate was seen ($P < 0.05$) with a range from 25.9 percent for the 100 µg/kg implant to 100 percent for the 75 µg/kg implant (Table 10). A significant difference in egg quality was also observed ($P < 0.05$) with the 75ug/kg treatment, 3.9, again being superior to the 100 µg/kg treatment, 3.0.

DISCUSSION

Early in the spawning season latency for implanted females and those with low dose injections tended to be longer than that for higher dose injections. As the season progressed and eggs had been exposed to an increasing number of temperature days, the latencies of injections and implants became very similar.

Higher doses of injections and implants, in general, tended to result in greater relative fecundity and greater numbers of eggs stripped from the females than lower doses of injections, and resulted in greater relative fecundity than lower doses of injections.

High dose implants and low dose injections adversely affected egg quality. By the peak of the spawning season, egg quality generated by implants was better than that from injections.

Indirect contact and, therefore, probable pheromone communication increased hatch rate and fry/kg by about 3X. Direct contact also had an inconsistent effect and lesser impact on these traits, increasing hatch 2X and fry production 1.5X in some cases. However, in some cases direct contact lowered fecundity and fry production by 40%.

Culturists producing hybrid catfish embryos have suspected that the first eggs stripped from a female are of the highest quality, egg quality decreases as one strips additional eggs and the last eggs stripped are of questionable use and viability. Most females produce 2-4 pans containing 100-150g of eggs each and some more. In 2004,

hatch of the first 4 pans was essentially equivalent (20.7-23.2% hatch), and then hatch rate rapidly decreased for pans 5-7, 14.0, 7.6 and 6.5%, respectively. Effects of egg mass order were more dramatic in 2005. The first 3 pans had 28.6-31.2 % hatch, pan 4 had 12.2 % hatch and pans 5-6 4.1-4.4% hatch. The first 3-4 pans of eggs should give consistent and good hatch. Quality of the last eggs stripped from large lots of eggs decreases. If hatchery space is a limitation, these eggs should be discarded or not stripped. If space is not a limitation, these eggs will hatch, but at a low rate, and benefit can still be attained.

The fry/kg produced by females ovulated in bags versus those loose in tanks was variable. In some cases bags appeared more effective and in other cases tanks appeared more effective. The females held in bags did not produce more fry per kilogram than those left loose in the tank. However, the bags provide a way to separate the females so the ovulating females can be immediately identified. There is less handling stress with the females in bags because the weighing only occurs one time. Though an improvement in fry production was not clearly demonstrated, minimal stress should benefit the spawning females and reduce labor requirements. Additionally, there may be an effect on post-spawning survival, which has not been examined yet.

Two treatments, 100µg/kg implants and 30/150 injections, yield the greatest number of fry/kg. Of those two, 100µg/kg implants was the most consistent treatment, and had the maximum mean fry/kg. A single injection of LHRHa was not effective compared to implants in regards to ovulation %, egg quality and fry/kg. Data for experiments late in the spawning season were incomplete, likely because of the use of poor quality sperm solutions. However, data of Kristanto (2004) and other unpublished

data from our laboratory, indicates that both the doses of the implants and injections must be reduced late in the spawning season when the eggs have been exposed to a maximum number of temperature degree days, and are close to the time for reabsorbance. Preliminary data indicates that 75µg/kg implant doses are the most effective treatment at this time to improve hatch rate and fry production. Late in the spawning season 75µg/kg implanted females had both higher ovulation rate and egg quality compared to females implanted with 100µg/kg of LHRHa.

Application of the best treatments examined in these experiments could result in commercial levels of hybrid fry production. Utilization of this hybrid would then have a positive impact on the economics of the American catfish industry (Ligeon et al. 2004abc).

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III. EFFECT OF LUTEINIZING HORMONE RELEASING HORMONE ANALOGUE IMPLANTS ON SPERM PRODUCTION IN THE MALE BLUE CATFISH (*Ictalurus furcatus*)AND ON THE PRODUCTION OF THE CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE CROSS BLUE CATFISH MALE HYBRID FRY

ABSTRACT

Luteinizing hormone release hormone analogue (LHRHa) implant has been successful in stimulating ovulation in channel catfish, *Ictalurus punctatus.* Using hormones to induce spermiation in blue catfish males for the production of the channel catfish female X blue catfish male hybrid has not been studied previously. The implants were given 48 hours before the testes were removed for the sperm.

Strain differences in sperm per gram of testes and sperm per kilogram of male were seen, but no significant differences ($P>0.05$) were seen between the implant and control treatments for these traits. However, in 4 of 5 strains, embryos of implanted males had higher observed mean hatch rates. Strain had larger effects than implantation. Large strain differences existed for sperm/g of testes, sperm/kg male body weight and hatching percentage.

The implantation appears to result in higher quality sperm with a greater number of the sperm mature and capable of fertilization. The application of LHRHa implants for blue catfish should result in both greater channel-blue hybrid catfish embryos, and a reduction in the number of blue males needed in a commercial hatchery producing hybrid embryos.

INTRODUCTION

The hybrid resulting from the mating of female channel catfish, x male blue catfish is the best catfish for pond culture (Dunham et al. 2001). Reproductive isolating mechanisms, behavioral, between blue catfish and channel catfish, have prevented consistent commercial scale production of hybrid catfish, although this is gradually changing as technology improves. The reproductive isolating mechanism is behavioral, and there are no gametic blocks for making this hybrid. The fertilization rate and the hatching rate of the channel x blue hybrid embryos and the fry production per kg of female are similar to the channel catfish pairs when the two genotypes are produced using the same technique (Bart 1994; Dunham et al. 1999; Lambert et al. 2000).

Artificial hand stripping and fertilization technologies have been developed that allow small-scale production of the channel-blue hybrid (Bart 1994; Dunham et al. 1999; Lambert et al. 2000). However, today only a few farmers utilize the hybrid because fingerlings are not available and because of the difficulty in making hybrid eggs and fry.

One inefficiency and obstacle to artificial fertilization in catfish is sperm concentration and availability. There has been limited success in stripping or ejaculating males(Dunham 1993) so the fish must be sacrificed to obtain sperm. This is especially wasteful since it takes 5 years to produce a sexually mature male blue catfish**.** Optimum fertilization procedures need to be developed that can overcome the need for so many blue catfish males

Inducing ovulation in female fish has been accomplished with many different compounds while using these chemicals to prepare the males is less common. However, hormone induction of males has sometimes yielded positive results. The use of hormones in males has shown that more viable sperm were produced by fish that were injected with hormones.

A single LHRH injection in paddlefish, *Polyodon spathula*, increased the number of sperm and the activity of the sperm (Lim et al. 2003; Cacot et al. 2003). In three year old starlet, *Acipenser ruthenus,* the evaluation of the effects of a single injection of Kobarelin or Ovopel, both mammalian GnRH analogues plus dopamine receptors, resulted in an increase in a higher concentration of spermiating males (Rzemieniecki et al. 2004). LHRHa has been the most effective compound for stimulating the gonadotropin release to induce spermiation in stellate sturgeon, *Acipenser stellatus* (Barannikova and Bukovskaya 1991).

Hormone injection is one option to improve male reproductive performance, and genetic enhancement is another option. Additionally, different genotypes of males could respond differentially to hormone treatment. Large strain differences have been observed for reproductive traits of channel catfish (Broussard and Stickney 1981). Females of the Norris and USDA-103 strains typically spawn at two years of age, whereas females of the Kansas and Marion strain are known to require at least a third year to reach sexual maturity. Other studies have suggested that different strains spawn at different ages, but no significant difference in spawning success (production of viable eggs) was evident (Broussard and Stickney 1981; Dunham et al. 1983b). No information is available on genetic variation for reproductive traits in blue catfish.

The ability to increase the density and viability of sperm per blue catfish for hybrid production has many benefits. Since the males are usually sacrificed for testicular removal, decreasing the number of fish sacrificed should reduce the cost of hybrid production. With increased production of viable sperm, the eggs that are fertilized should have a higher hatch rate.

The objective of the experiment was to evaluate the effect of LHRHa implantation on sperm production and hatch rate of hybrid catfish embryos in different strains of blue catfish male. The overall objective is to increase the efficiency of channel-blue hybrid catfish fry production.

MATERIALS AND METHODS

The experimental fish were held at the North Auburn Fisheries Station. The males and females were held in ponds until they were moved to tanks or spawning pens within a pond to begin spawning preparation. The females were selected by strain and by the size of the belly and the redness of the genital opening.

The females for spawning were held in tanks measuring 3.0 x 0.47 x 0.61 m and with a water volume from 670 – 837 liters. Each tank had a constant flow through and compressed air for aeration.

Five strains of blue catfish males were used designated AU1, AU2, AU3 AU4 and AU5. Each strain had a treatment group receiving an implant and a control group receiving no implant. The treatment groups contained 2 to 4 fish each, depending on the number of males available. The males were implanted and then moved into spawning pens in a pond for the duration of their latency. The fish were then retrieved from the pens and brought into tanks to await testes removal.

Sperm Preparation

During the experiment, the males were given a 100µg/kg LHRHa implant three centimeters from the top of the fish just behind the dorsal fin. The experimental fish were given a 48 hour latency period. The males were sacrificed or surgery was done on a male anesthetized in a solution with 200 ppm MS 222 and 200 ppm sodium bicarbonate until their movement slowed. They were then cut along the center of their abdomen with

a scalpel. The testes were removed and then the incision was stapled with surgical staples. On some of the fish had only a single testis removed while for other fish both testes were removed. The fish received a permanganate treatment to prevent infection and were returned immediately to a 1011.7 square meter pond for recovery. Weights were recorded for all of the males. When all of the sperm was released from the testicular tissue, 10 ml of salt 0.9% saline per g testes was used to remove all tissue. The solution was strained with a 0.5millimeter mesh two times to remove all excess tissue and blood. The solution was then placed in a beaker. The beakers were covered loosely so that there was air circulation. A total of 6.5×10^7 sperm per one hundred eggs were used for fertilization. The amount of sperm per milliliter of solution was determined by diluting the sperm sample 10 to 50 times and then determining absorbance of the sample with spectrophotometer at 546 nanometers. A graph was constructed. The numbers were then plotted so that the number of sperm could be calculated from the spectrophotometer reading.

Female Spawning Preparation

Twenty female channel catfish were divided into two treatment groups. The females were given a 75 or a 100 implant. All of the females were held in bags. The average temperature was 26.3 ° C. Each of the females was stripped and the eggs were placed in a series of pie pans. Each pan was fertilized with sperm from one male. Egg masses were transferred to hatching troughs by male and female treatment group.

Implants

To induce ovulation, the females received a single LHRHa implant. The implants were given in a single dose just behind the dorsal fin and ventrally down the body approximately 3cm.

Artificial spawning

Ovulation occurred approximately 40 to 55 hours after the priming dose of the injections and 45 to 60 hours after implants. All females were checked for eggs every 3 hours after the first fish gave eggs. The females were checked for eggs by gently pushing on the abdomen and rubbing gently from head to tail. If the females were not ovulating they were returned to their tank and checked again. Bags were checked for eggs visually or by gently lifting and moving the bag to minimize the disturbance of the female. If eggs were present on the bag, the fish and the bag were removed from the water and placed in the anesthesia. When the females were giving eggs freely, they were placed in a solution with 200 ppm MS 222 and 200 ppm sodium bicarbonate until their movement slowed. The fish were then dipped into a tank of freshwater while the vent was covered by a finger to keep the eggs from leaking out while rinsing off the anesthesia. The fish was placed on a dry towel and the head was covered with a towel to catch any water leaking from the gill cavity. The fish were then taken to the stripping table and hand stripped. The eggs were stripped into pie pans greased with a thin layer of vegetable shortening to prevent sticking. The females were stripped of eggs until the eggs no longer flowed. The pie pans generally were filled with 70-150g of eggs and then a new pan was started so that all females had several egg masses.

Fertilization

The eggs were fertilized within minutes of the eggs being stripped and weighed. The eggs were rinsed with saline solution to remove all the blood and excess tissue from the female. If no blood was present, the eggs were not rinsed. The sperm solution was drawn into a 1 ml or 3 ml syringe. A total of 6.5×10^7 sperm per one hundred eggs were used for fertilization.

The sperm was added to the egg mass in a circular motion to expose all the eggs to the sperm solution. Dechlorinated water was then added to the egg mass and sperm solution in the pie pan to activate the eggs and begin the fertilization process. The egg and sperm were gently swirled together. The fertilized egg masses were then allowed to sit for 2 to 10 minutes until they formed a mass and were then transferred to a water hardening trough. The eggs remained in the water hardening trough for at least 15 minutes. In the water hardening trough there was constant water flow and aeration. The eggs were transferred to an egg basket in a paddle wheel hatching trough. The troughs had an air supply and a paddle wheel which was turned on when the youngest egg mass in the trough was at least 3 hours old.

Incubation

The eggs were held in tanks with paddlewheels until hatch. The eggs treatment began 12 hours after they were fertilized. The initial treatment was always formalin and then the eggs were treated three times a day, the first with 32 ppm copper sulfate, the second with 100 ppm formalin, and the last with copper sulfate to help prevent fungus growth until they began to hatch. The eggs were not treated between 42 to 46 hours at

28ºC after fertilization because that was found to be a critical period of development adversely affected by formalin treatments (Small and Chatakondi, 2006).

Data Analysis

Number of sperm per gram of testes, number of sperm per kilogram of male, and hatching rate were calculated for each treatment group. Statistical analysis of data was conducted using Statistical Analysis System 9.1 (SAS Institute 2004). Analysis of Variance was used to determine the effects of genotype, treatment and their interaction. A Duncan's Multiple Range Tests (DMRT, Duncan 1955) was used to compare means of variables that showed significant response to genotype or treatment at the 0.05 level of significance.

RESULTS

Sperm Density

Males implanted with LHRHa did not (0>.05) have more sperm than the controls. However, in the AU1 strain there was significantly more sperm for implanted males than the control males of the same strain (Table 11). Observed means for implanted males sperm/ g testes and sperm/ kg male body weight were variably larger, similar and smaller compared to controls among strains. Strain differences ($P<0.05$) existed for number of sperm per gram of testes and number of sperm per kilogram of male (Table 12).

Percent Hatch

When comparing implanted and non-implanted males within a strain there was a difference in percent hatch ($P<0.05$) between the AU4 strain blue males that were not implanted compared to the AU4 implanted males (Table 11).

Table 11. Sperm/g of testes, sperm/kg of body weight and hybrid embryo hatching rates for blue catfish, *Ictalurus furcatus,* males with and without 100µg/kg implants of GMP grade LHRHa, sacrificed 48 hours after implantation and mated with channel catfish,*I. punctatus* females

Strain	Treatment	N	Average Sperm/g of Testes ($X10^8$)	Average Sperm/kg of Male ($X10^8$)	Percent Hatch
AU5	Implant	3	$9.8^{b,c,d}$ ± 3.6	$12.5^{b,c,d,e}$ ± 1.6	1.8^{b} ± 2.5
AU5	Non	2	$10.9^{b,c,d}$ ± 4.4	19.7^{a} ± 23.2	0.6^{b} ± 1.6
AU4	Implant	4	$11.4^{a,b,c}$ ± 4.5	$15.2^{a,b,c}$ ± 1.8	17.1^{b} ± 24.0
AU4	Non	4	$11.4^{a,b}$ ± 4.8	$17.8^{a,b}$ ± 4.2	52.7^{a} ± 34.3
AU1	Implant	3	14.5^{a} ± 6.4	$7.5^{c,d,e}$ ± 7.6	3.2^{b} ± 3.0
AU1	Non	3	7.8^{d} ± 1.9	$7.8^{d,e}$ ± 5.5	2.0^{b} ± 2.4
AU2	Implant	3	$11.3^{a,b,c,d}$ ± 15.2	$12.7^{a,b,c,d}$ ± 9.7	10.6^{b} ± 21.8
AU2	Non	3	$12.8^{a,b}$ ± 4.8	6.4^{e} ± 2.7	10.3^{b} ± 8.3
AU3	Implant	3	$8.6^{b,c,d}$ ± 5.8	$7.4^{d,e}$ ± 1.2	12.3^{b} ± 13.6
AU3	Non	3	$8.2^{c,d}$ ± 1.6	6.2^{e} ± 3.4	9.2^{b} ± 15.9

a,b,c, means followed by the same letter are not different (P>0.05) within each column

Table 12. Sperm/g of testes and sperm/kg of body weight for blue catfish, *Ictalurus furcatus,* males with and without 100µg/kg implants of GMP grade LHRHa, sacrificed 48 hours after implantation and mated with channel catfish, *I. punctatus* females.

Strain	N	Average Sperm/g of Testes ($x10^8$)	Average Sperm/kg of Male ($x10^8$)	Percent Hatch
AU1	6	$11.1^{a,b,c} \pm 3.5$	$9.4^{b} \pm 5.4$	$2.6^{b} \pm 2.7$
AU2	6	$11.8^{a,b} \pm 3.2$	$9.9^{b} \pm 7.1$	$10.4^{b} \pm 15.8$
AU3	6	$9.0^{c} \pm 3.7$	$6.5^{b} \pm 2.0$	$10.9^{b} \pm 14.3$
AU4	8	$12.2^{a} \pm 4.3$	$16.7^{a} \pm 33.3$	$26.1^{a} \pm 31.8$
AU5	5	$9.4^{b,c} \pm 2.8$	$15.5^{a} \pm 10.6$	$1.3^{b} \pm 2.2$

a,b,c, means followed by the same letter are not different (P>0.05) within each column

DISCUSSION

Genetics appears important as the responding strains, AU-1 and -3 are closely related. Sperm/kg BW increased in 3 of 5 strains with a pooled increase of almost 20%. LHRHa implants appear to have potential to increase sperm production in blue catfish, but individual variation is so great that larger experiments are needed to verify these apparent differences. Genotype – environment interactions exist, and the implants appear effective in some, but not all strains.

The most dramatic effect of implanting blue catfish males was on hatching rate of the hybrid embryos In 4 of 5 strains, embryos of implanted males had observed higher hatch rates.

Strain had larger effects than implantation. Large strain differences existed for sperm/g of testes, sperm/kg male body weight and hatching percentage.

The use of LHRHa implants for induced spermiation has potential to increase the number of sperm. Coupled with strain selection, sperm production could be increased. LHRHa could also allow for the males to have increased testicular development if done over a more extensive period of time, and this should be evaluated and contrasted to the short term effects reported here.

The implantation apparently results in higher quality sperm with a greater number of the sperm mature and capable of fertilization. The application of LHRHa implants for

blue catfish should result in both greater channel-blue hybrid catfish embryos, and a reduction in the number of blue males needed in a commercial hatchery producing hybrid embryos.

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