SOUTHERN REGIONAL
AQUACULTURE CENTER

Annual Progress Report
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IMPROVING CATFISH BROODSTOCK MANAGEMENT BY MANIPULATING DIET, STOCKING DENSITIES AND SEX RATIOS

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	Year 2	$127,400
	Year 3	$145,125
	Total	$388,385

Participants	University of Arkansas at Pine Bluff	Rebecca Lochmann, Carole Engle, Alf Haukenes
	Texas A&M University	Camilo Pohlenz, Delbert M. Gatlin, III
	USDA-ARS Warmwater Aquaculture Research Unit	Brian Bosworth, Sylvie Quiniou, Geoff Waldbieser

PROJECT OBJECTIVES

1. Identify diet formulations to improve reproductive performance (egg biochemical composition, fecundity, egg and fry quality) of catfish and determine associated effects on production costs.

    a. Manipulate dietary protein concentration and lipid sources and assess reproductive performance and diet effects on production costs (Tank Trial 1).
    b. Refine dietary protein and lipid sources, add nucleotides, and assess reproductive performance and diet effects on production costs (Tank Trial 2).
    c. Conduct concurrent pond trials at UAPB and TAMU to assess reproductive performance and diet effects on production costs using a high-performance diet and an economical diet (based on results of sub-objectives 1 a and 1 b, above) and a standard commercial diet.

2. Determine effects of sex ratios, stocking densities, and post-spawning brood-fish consolidation on catfish reproductive success and determine associated costs.

    a. Effects of broodfish sex ratios.
    b. Effects of broodfish stocking rates.
    c. Effects of post-spawning broodfish consolidation.

ANTICIPATED BENEFITS

Development of diets or supplements specifically designed to meet the requirements of catfish broodstock should optimize spawning performance and ensure the production of high-quality eggs and fry. Information on the effects of dietary lipids, proteins, and nucleotides on gamete and fry production and quality could reduce the number of broodfish needed to meet production goals. Altering traditional management protocols by using different sex ratios or stocking densities could improve economic efficiencies of fry production and improve profitability for farmers. Research in catfish has just begun to yield data sufficient to support detailed economic analysis of broodstock and hatchery performance and management practices in the U.S.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Identify diet formulations to improve reproductive performance (egg biochemical composition, fecundity, egg and fry quality) of catfish and determine associated effects on production costs.*

Sub-objective 1a. *Manipulate dietary protein concentration and lipid sources and assess reproductive performance and diet effects on production costs (Tank Trial 1).*

University of Arkansas at Pine Bluff; Texas A&M University

Industry wide, only 30 to 40% of female catfish spawn each year. The reasons for the poor spawning performance are not clear, so producers maintain an excess of broodstock to ensure that egg production goals are met. Development of diets or supplements to diets specifically designed to meet the requirements of catfish broodstock may optimize spawning performance and ensure the production of high-quality eggs and fry.

Four experimental diets (Table 1), with simple modifications to protein concentration and lipid sources, were formulated based upon results of earlier channel catfish broodstock nutrition research conducted by the participating universities. These diets were formulated to provide effective broodstock nutrition for catfish produced throughout the southern region and incorporated economic considerations and dietary modifications based upon other research on this topic. The four diets were evaluated (4 replicates/diet; stocking ratio of 1:3 male to female) using physical and biochemical methods including analyses of egg composition, egg production and enumeration, and egg and fry quality.

Table 1. Formulae of diets for Trial 1, sub-objective 1a (2012) in the Southern Regional Aquaculture Center catfish broodstock management study (%, as-fed). Diets were extruded at the Harry K. Dupree Stuttgart National Aquaculture Research Center.

Ingredient (%)	Diet 1- control (36% protein)	Diet 2 (36% protein)	Diet 3 (32% protein)	Diet 4 (32% protein)
Menhaden fish meal	6.0	-	3.5	–
Poultry by-product meal	10.0	6.0	7.5	4.0
Meat/bone/blood meal, pork	-	6.0	-	4.0
Soybean meal	49.0	54.0	38.0	41.0
Cottonseed meal	6.0	6.0	8.0	8.0
Corn starch	20.0	19.0	18.8	18.8
Wheat middlings	0.0	0.0	16.0	16.0
Menhaden fish oil	4.0	4.0	3.6	3.6
Poultry fat	4.0	4.0	3.6	3.6
Vitamin premix[a]	0.48	0.48	0.48	0.48
Stay-C	0.02	0.02	0.02	0.02
Mineral premix[a]	0.50	0.50	0.50	0.50

[a] Standard vitamin and mineral premixes used at UAPB.

There were no differences in water temperature at the time of spawning (Mean ± SE; 77.5 ± 1.0 degrees F), individual egg weight (0.031 ± 0.00 oz), quantity of eggs per milliliter (24.2 ± 1.4 eggs), quantity of eggs per spawn (18,944 ± 2,674 eggs), hatch rate (87.5 ± 2.9 %), fry survival to 14 days (92.0 ± 2.3 %), fry produced per 1,000 eggs (801 ± 36), or feed required to produce 1,000,000 fry (643 ± 233 pounds). Spawning success during the study was considered poor, but within the industry average (30-40%). Spawning success for females fed diets 1 and 3 (41.6%) was greater than those of females fed diets 2 and 4 (33.3%). Total egg mass weight (matrix intact) was greatest for fish fed diet 1, followed by diet 3, diet 2, and diet 4 (1.77, 1.61, 1.06, and 0.98 pounds, respectively). The total egg volume (matrix removed) was greatest for fish fed diet 1 (65.5 in^3) and lowest for diet 4 (33.7 in^3), while diets 2 (37.1 in^3) and 3 (54.3 in^3) were intermediate. The total quantity of 14-day-old fry produced was greater for fish fed diet 1 (118,257 fry) than for fish fed diets 2 and 4 (43,173 and 47,579 fry, respectively), while fry from fish fed diet 3 were intermediate (78,438 fry). The total quantity of 14-day-old fry produced per female was greater for fish fed diet 1 (9,854 fry) than for fish fed diets 2 and 4 (3,597 and 3,964 fry, respectively), while fry from fish fed diet 3 were intermediate (6,536 fry). The total pounds of females needed to produce 1,000,000 fry were significantly lower for diets 1 and 3 (5,562 and 8,158 pounds, respectively) than for diets 2 and 4 (15,382 and 12,564 pounds, respectively). The pounds of feed needed to produce 1,000,000 fry were significantly lower for diet 1 (3,392 pounds) than for diets 2 or 4 (9,381 and 7,665 pounds, respectively), while diet 3 was intermediate (4,976). There were no significant differences in amino acid composition of the eggs produced by fish fed the various diets. Fatty acid analysis of the eggs is ongoing. A standard cost analysis was developed for the various broodfish diets developed, in terms of both egg and fry production. The least expensive diet, Diet 4 (32% protein - no added nucleotides), was identified to be used in the followup study.

Diet 1 (containing 36% protein and menhaden fish meal) performed the best in terms of fry production, so it was also included in the followup study. Further evaluation of diet 1 (the most expensive) is warranted to determine if it will reduce the amount of feed and the total pounds of females required to achieve the most efficient production of channel catfish fry compared to 32% protein diets and diets that replace fish meal with other animal by-products.

Sub-objective 1b. *Refine dietary protein and lipid sources, add nucleotides, and assess reproductive performance and diet effects on production costs (Tank Trial 2).*

University of Arkansas at Pine Bluff; Texas A&M University

The diet from sub-objective 1a that resulted in the best reproductive performance and fry production (Diet 1: 36% protein- 6% menhaden fish meal) and the diet with the lowest production costs (Diet 4: 32% protein - no added nucleotides) were carried through to 2013 for sub-objective 1b and evaluated against two new experimental diets (Table 2). New diet formulations were based on the best performing diets from sub-objective 1a, but contained less animal protein, more cost-saving plant proteins, and a yeast nucleotide supplement.

The four diets were evaluated (4 replicates/diet; stocking ratio of 1:3 male to female) and performance indices describing fish survival and egg production were used to characterize the response to diets.

In 2013, a high incidence of fungal infections during incubation led to the majority of the spawns being lost. Due to the low frequency of success during the incubation period, hatch rate and fry survival rate were not included in our characterization of response to the diets. Female catfish survival rates ranged from 50-92%. However, the high variation associated with tanks within treatment led to no significant differences among diets (P = 0.32). The highest percent survival was observed in fish fed Diet 4 (92%). Percent survival for females fed Diets 1, 2, and 3 were 50, 50, and 58%, respectively. Spawning success during the study was considered poor, 33%, but within the industry average (30-40%). Spawning frequency among fish fed the experimental diets approached our threshold of significance (P = 0.07). The highest frequency of spawning was observed in fish fed Diets 3 and 4 (50.0%) and the lowest frequency of spawning was observed in fish fed Diet 2, 8.3%. The average total egg mass weight (matrix intact) was greatest for fish fed Diet 4, followed by Diet 1, and Diet 3 (1.26, 1.14, and 0.79 pounds, respectively). The total egg volume (matrix removed) was greatest for fish fed Diet 4 (37.0 in^3) and lowest for Diet 2 (1.8 in^3), while Diets 1 (33.0 in^3) and 3 (29.3 in^3) were intermediate. Although few statistical differences were noted due to small sample sizes and/or high variability in the data, Diet 4 (32% protein - no added nucleotides) generally performed better than the other 3 diets based on female percent survival, spawning frequency and spawn size. Additional statistical analysis of results is pending, but Diet 4 was also the least expensive diet and will be tested in the pond trial (sub-objective 1c) in 2014. Fatty acid and amino acid analysis of eggs is in progress.

Table 2. Formulae of diets for Trial 2, sub-objective 1b (2013) in the Southern Regional Aquaculture Center catfish broodstock management study (%, as-fed). Diets were extruded at the Texas A&M University Food Protein R&D Center.

Ingredient (%)	Diet 1 control (36% protein)	Diet 2 (36% protein)	Diet 3 (32% protein+ GroBiotic®-A)	Diet 4 (32% protein - no GroBiotic®-A)
Menhaden fish meal	6.0	-	-	-
Poultry by-product meal	10.0	4.0	4.0	4.0
Meat/bone/blood meal, pork	-	4.0	4.0	4.0
Soybean meal	49.0	52.0	41.0	41.0
Wheat gluten	-	2.0	2.0	-
Cottonseed meal	6.0	10.0	10.0	8.0
Corn starch	20.0	19.0	18.8	18.8
Wheat middlings	-	-	10.0	16.0
GroBiotic®-A[a]	-	-	2.0	-
Menhaden fish oil	4.0	4.0	3.6	3.6
Poultry fat	4.0	4.0	3.6	3.6
Vitamin premix[b]	0.48	0.48	0.48	0.48
Stay-C®[c]	0.02	0.02	0.02	0.02
Mineral premix[b]	0.50	0.50	0.50	0.50

[a] GroBiotic®-A (International Feed Ingredients, Corp., St Louis, MO) was used as a nucleotide source.
[b] Standard vitamin and mineral premixes used at UAPB.
[c] Stay-C®35 (DSM Nutritional Products LLC, Parsnippany, NJ).

Sub-objective 1c. *Conduct concurrent pond trials at UAPB and TAMU with a high-performance diet, an economical diet (based on tank trials), and a standard commercial diet, and assess reproductive performance and diet effects on production costs.*

University of Arkansas at Pine Bluff; Texas A&M University

On schedule to begin in February/March 2014. Diets will include Diet 1 (36% protein with fish meal), Diet 4 (32% protein - no added nucleotides), and a third diet with 32% protein and a higher inclusion rate (4%) of the nucleotide source tested in sub-objective 1b.

Objective 2. *Determine effects of sex ratios, stocking densities, and post spawning broodfish consolidation on catfish reproductive success and determine associated costs.*

Sub-objective 2a. *Effect of broodfish sex ratios.*

USDA-ARS Warmwater Aquaculture Research Unit

Commercial catfish farming is the largest commercial aquaculture enterprise in the U.S. However, catfish production in the U.S. has decreased approximately 50% over the last 10 years due to increased production costs and competition from imported farmed catfish and *Pangasius*. In order to remain competitive in a global market, U.S. catfish farmers must reduce production costs. Refinement of catfish broodfish management strategies could improve reproductive efficiency and reduce production costs. Currently most farmers use a 1:1 or 1:2 male to female stocking ratio in brood ponds. However, unpublished data from the USDA-ARS Catfish Genetics Research Unit has demonstrated that less than 10% of the males present account for over half the spawns, and about half of the males do not spawn at all. Therefore, commercial producers could possibly improve spawning efficiency by reducing the biomass of males in brood ponds and replacing males with additional females. Costs of maintaining broodfish (feed, pond space, etc.) would be similar regardless of sex ratio if the biomass was constant. Previous published data demonstrated no differences in the percent of females spawning at 1:1 and 1:4 male to female ratios, although study ponds were unreplicated. The objective of this study was to determine the effects of broodfish sex ratios on channel catfish spawning success and associated costs.

Mature Delta Select strain channel catfish males and females (3 and 4 years old, respectively) were randomly stocked at about 1000 pounds per acre in 0.25 acre ponds during the last week of February, 2012. Individual broodfish had been marked previously with pit tags and sampled for DNA to be used for subsequent parentage determination. Two sex ratios were compared: a 1:1 male to female ratio (30 males and 30 females per pond) and a 1:4 male to female ratio (12 males and 48 females per pond) with six replicate ponds per treatment. Spawning cans were placed in ponds the second week of March at a rate of 2 cans for every 3 males (8 cans in the 1:4 ponds and 20 cans in the 1:1 ponds). One week after placing spawning cans in ponds, they were checked 2 to 3 times per week for spawns through mid-July. Spawns were removed, brought to the hatchery, and weighed. A sample of eggs was taken from each spawn, weighed and counted, and the counts were used to determine total eggs per spawn. Each spawn was hatched in a separate 20-gallon fiberglass tank provided with flow-through ground water (1 gal/min, 79 degrees F, 5 ppm D.O.). Eggs were treated with hydrogen peroxide once daily until the eyed stage. Sac fry were siphoned into a volumetric cylinder and number of fry was determined volumetrically. Ten to twenty fry were sampled from each spawn, preserved in ethanol, and used for DNA isolation for parentage determination. Parents and fry were genotyped for 2, multiplexed DNA microsatellite panels to determine the individual male and female parent of each spawn. Details of protocols used for parentage determination are given by Waldbieser and Bosworth (Animal Genetics, accepted). In August 2012, ponds were seined and drained and remaining fish were counted and pit tags were recorded to allow determination of broodfish survival. At the time of this report, DNA determination of parentage was still underway; therefore, we do not present data on individual female and male spawning success. We assumed each spawn was produced by a single female in estimates of female spawning percentage. Information on individual spawning success and number of spawns produced by individual male and female broodfish will be included in the final report when the parentage analysis is complete. The broodfish sex ratios were compared by ANOVA for broodfish survival, percent of females spawning, spawning day (the first day a spawn was collected was defined as day 1 and all subsequent spawn dates were determined relative to day 1), spawn weight, percent hatch, number of spawns per acre of brood pond, weight of eggs per acre, number of eggs per acre, and number of fry per acre.

Reproductive traits for 1:4 and 1:1 male to female channel catfish broodfish treatments are summarized in Table 3. Male broodfish had lower survival than female broodfish (65.5 versus 91.4%) but there was no effect of sex ratio on survival of males or females. Spawning date, spawn weight, and percent hatch were not affected by broodfish sex ratio. Percent of females spawning was over 3 fold higher for the 1:1 male to female ratio compared to the 1:4 ratio (57.2% versus 16.3%). The much higher percentage of females spawning at the 1:1 male to female ratio resulted in the 1:1 ratio being superior to 1:4 even when reproductive traits were considered on a per acre basis. Relative to the 1:4 ratio, the 1:1 ratio resulted in more spawns per acre, greater weight and number of eggs produced per acre, and greater number of fry per acre.

Table 3. Influence of channel catfish broodfish sex ratios (1:4 vs.1.1 male to female ratio) on broodfish survival and reproductive traits.

Treatment Male:Female	Broodfish Survival (%) Male	Broodfish Survival (%) Female	Females Spawning Incidence (%)	Spawning Date (Days)	Spawn Weight (lbs)	Hatch (%)	Spawns per acre (#)	Weight of eggs per acre (lbs)	Eggs per acre (#)	Fry per acre (#)
1:4	66.7	89.2	16.3[a]	33.0	1.0	28.3	31[a]	31.1[a]	343130[a]	102500[a]
1:1	64.2	93.5	57.2[b]	30.6	1.1	29.9	69[b]	72.8[b]	801840[b]	232240[b]
SE	7.5	6.3	6.9	4.5	0.12	6.1	10	9.7	110688	40543
P value	0.750	0.509	0.002	0.590	0.510	0.800	0.024	0.013	0.011	0.047

The results demonstrate that a greater percentage of channel catfish females spawned at a 1:1 male to female broodfish ratio than at a 1:4 male to female ratio. The differences in female spawning percentage were large enough that the 1:1 male to female ratio was superior to the 1:4 ratio even when reproductive output was considered on a per acre basis. The increased number of females per acre in the 1:4 ratio ponds would have had an advantage on a per acre basis if the percent of females spawning had been similar in the two treatments. Therefore, the results of this study indicate the reproductive efficiency and economics of channel catfish fry production at a 1:1 male to female broodfish ratio are superior to those of a 1:4 male to female ratio.

Sub-objective 2b. *Effects of broodfish stocking rates.*

USDA-ARS Warmwater Aquaculture Research Unit

Currently most farmers stock broodfish at about 1,000 pounds per acre with a 1:1 or 1:2 male to female stocking ratio in brood ponds. However, increasing stocking density would decrease labor for broodfish feeding and pond management as these costs are primarily influenced by the number or acres of ponds stocked than by the stocking density. In addition, reducing the acres devoted to broodfish ponds would make more pond area available for fingerling production or allow unused acres to be converted to row-crop or other uses. Therefore, commercial producers could possibly improve spawning efficiency by increasing the stocking density in brood ponds. The objective of this study was to determine the effects of broodfish stocking density on channel catfish spawning success.

Mature Delta Select strain channel catfish males and females (4 and 5 years old) were randomly stocked in 0.25 acre ponds during the last two weeks of February, 2013. Broodfish had been previously individually marked with pittags and sampled for DNA to be used for subsequent parentage determination. The original design was to stock broodfish at 1,000 and 2,000 pounds per acre (low and high density, respectively) but actual stocking densities were 951 and 1829 pounds/acre, respectively. Five ponds were stocked for each treatment at sex ratios of 1.8 to 1 females to males (30 females and 17 males in the low density, 60 females and 34 males in the high densities. Spawning cans were placed in ponds the second week of March at a rate of 1 can for every 3 females (10 cans per pond in the low density and 20 cans per pond in the high density treatments). One week after placing spawning cans in ponds, they were checked 2 to 3 times per week for spawns through mid-July. Spawns were removed, brought to the hatchery, and weighed. A sample of eggs was taken from each spawn, weighed and counted, and the counts were used to determine total eggs per spawn. Each spawn was hatched in a separate 20 gallon fiberglass tank provided with flow through ground water (1 gal/min, 79 degrees F, 5 ppm D.O.). Eggs were treated with hydrogen peroxide once daily until the eyed stage. Sac fry were siphoned into a volumetric cylinder and number of fry was determined volumetrically. Starting in the third week of July, 2013, ponds were seined and drained and remaining fish were counted and pittags were recorded to allow determination of broodfish survival. We assumed each spawn was produced by a single female in estimates of female spawning percentage. Broodfish stocking densities were compared by ANOVA for broodfish survival, percent of females spawning, spawning day (the first day a spawn was collected was defined as day 1 and all subsequent spawn dates were determined relative to day 1), spawn weight, percent hatch, number of spawns per acre of broodfish pond, weight of eggs per acre, number of eggs per acre, number of eggs per pound of broodfish, number of fry per acre, and number of fry per pound of broodfish.

Survival and reproductive traits for low and high density broodfish stocking densities are summarized in Table 4. Male broodfish had lower survival than female broodfish (54.1% vs. 75.5%) but there was no effect of stocking density on male or female survival. Spawning date, spawn weight, and percent hatch were not affected by broodfish stocking density. Percent of females spawning was approximately 2 times higher at the high stocking density compared to the low stocking density (34.0% vs. 16.7%). The higher percentage of females spawning in the high density treatment resulted in high density treatment being superior for number and total weight of spawns and for eggs and fry produced per acre. However, eggs and fry per pound of broodfish did not differ significantly between treatments even though the eggs per pound of broodfish and fry per pound of broodfish were 89% and 42% higher respectively at the high density compared to the low density. The lack of statistical significance in eggs and fry per pound of broodfish was likely due to a combination of larger egg masses and higher percent hatch in the low density treatment, along with large standard errors due to the high variability among ponds within treatments for these variables.

Table 4. Influence of channel catfish broodfish stocking density (high = 1829 lbs/acre, low = 951 lbs/acre) on broodfish survival and reproductive traits.

Broodfish Density	Broodfish Survival (%)		Females Spawning Incidence (%)	Spawning Date (Days)	Spawn Weight (lbs)	Hatch (%)	Spawns per acre (#)	Weight of eggs per acre (lbs)	Eggs per acre (#)	Eggs per lb of broodfish	Fry per acre (#)	Fry per lb of broodfish
	Male	Female										
High	52.4	76.3	34.0[a]	33.0	1.30	29.7	81.6[a]	106.3[a]	332448[a]	676.9	1211212[a]	182.6
Low	56.0	74.7	16.7[b]	30.6	1.47	39.3	20.0[b]	29.4[b]	124056[b]	357.5	342671[b]	129.0
SE	4.2	3.1	4.4	4.5	0.13	5.4	10	18.7	53268	172.4	223049	50.0
P value	0.715	0.558	0.0001	0.590	0.195	0.075	0.0007	0.0034	0.0045	0.101	0.0046	0.315

The results demonstrate that a greater percentage of channel catfish females spawned at the high density (1829 pounds/acre) than at the low density (951 pounds/acre) leading to a greater weight of spawns, number of eggs, and number of fry produced per acre at the high stocking density. Although the eggs and fry per pound of broodfish did not differ significantly among treatments, from a catfish farmer's perspective the larger number of eggs and fry produced per pound of broodfish at the high density are probably meaningful. Therefore, the results of this study indicate the reproductive efficiency and economics of channel catfish fry production at the high stocking density were superior to a low stocking density. The biological basis for the higher spawning incidence of females observed at the high stocking density is not obvious. We expected, at best, that the higher stocking density would have a similar spawning incidence to the low stocking density. One possible explanation is the lower number of males per acre at the low stocking density. In the first year of this study, females stocked at a 1:4 male to female ratio had a much lower spawning percentage than females stocked at a 1:1 male to female ratio. Past research at our facility has demonstrated that approximately 10% of males are responsible for 60% of the spawning and 40% of males don't spawn at all. Therefore, given the high mortality and low initial number of males per acre (68) at the low stocking density it is possible that there were not enough males participating in spawning, which resulted in a lower incidence of females spawning. More research on the influence of male density and participation in spawning on incidence of female channel catfish spawning is needed.

A series of 2-year mathematical programming models have been developed for five different catfish farming initial conditions, tested, and validated. These models include both fingerling and foodfish growout options and will be used to evaluate the economic effects of various broodfish stocking rates on the farm level, under a series of farming conditions.

IMPACTS

Diet modification so far has had few clear effects on improving fry production efficiency, which may allow the use of lower-cost diets with more plant ingredients. Use of a 1:1 ratio of male to female broodfish significantly increased reproductive efficiency of channel catfish compared to standard commercial practices. A higher percentage of females spawned at the higher stocking density. Economic models have been developed to clarify the cost-effectiveness of the different feeding and management strategies.

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

Nothing to report.

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