

Effects of Varying Dietary Protein Levels and Feeding Frequencies on Condition and Reproductive Performance of Channel Catfish to Produce Hybrid Catfish

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Abstract

The interspecific hybridization of channel catfish, *Ictalurus punctatus*, females with blue catfish, *I. furcatus*, males has been identified as a method to further improve production; however, lack of spawning success has affected its commercial application. To facilitate our understanding of the interaction of brood stock nutrition and reproductive performance, we evaluated the interaction of feed quality and feeding frequency. Channel catfish females were classified into two genetic groups, namely, high and low spawning. The treatments were offered during the spring season 70–90 d prior to the start of the spawning season. Induced reproduction was performed using luteinizing hormone releasing hormone analog. Condition of the fish as well as reproductive performance using spawning success, egg production, egg size, and fertilization at 48 h were determined. Changing protein level of the diet from 32 to 42% did not influence spawning, fecundity, or fertilization, but affected egg size and biochemical composition of the eggs. Increasing the feeding frequency from three to six times per week negatively affected spawning in one of the two genetics groups, did not affect egg production and egg fertilization, but had a significant effect on egg size. Older fish performed better than younger fish in terms of spawning success and egg production.

The catfish industry in the USA started in the late 1960s, and has grown rapidly to become the largest segment of the aquaculture industry in the USA (Quagraine and Engle 2002; Engle 2003). Commercial catfish production generated over 42% of the total value (462 million dollars) of aquaculture production in the USA during 2005 (USDA 2006). Since 2000, the US farm-raised catfish industry has suffered because of low prices in the marketplace caused by the influx of imported fish, depressed prices for competing meats, and a weak economy (Hanson et al. 2004). These economic challenges will require developing new production technologies, market infrastructure, and market development (Engle 2003;

Hanson et al. 2004) that lead to enhanced productivity.

Hybridization techniques have identified the interspecific hybrid channel catfish, *Ictalurus punctatus*, female × blue catfish, *I. furcatus*, male as the most suitable for culture conditions, because of its better growth, higher resistance to low dissolved oxygen levels, resistance to diseases, ease of harvesting, and higher carcass yield (Dunham et al. 1987, 1990; Jeppsen 1995; Argue et al. 2003). However, reproductive problems have limited its application on a commercial scale (Tave and Smitherman 1982; Dunham and Smitherman 1987). Increasing interest in developing techniques to manipulate and control hybrid production has shown a significant increase during the past 25 yr, in areas such as physiological responses, genetic aspects, artificial fertilization, and husbandry

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methods (Dunham and Argue 2000; Kristanto 2004; Hutson 2006). However, a lack of information in nutritional status of brood stock is common, not only in channel catfish but also in other aquaculture species. This lack of information is in part because of the need for appropriate indoor and/or outdoor culture facilities for maintaining large groups of adult fish and the associated costs with those requirements (Izquierdo et al. 2001). Nutritional aspects of brood stock channel catfish were considered by Santiago (1979), who studied the effects of feeding and age on reproductive performance. Also, Abidin et al. (2006) evaluated the influence of dietary protein levels on egg quality in brood stock female bagrid catfish *Mystus nemurus* (Cuv. and Val.) The objective of this research was to evaluate the effect of dietary protein levels and frequency of feeding on egg performance of channel catfish females to produce hybrid catfish. Strain as genetic component, age as physiological component, and period of spawning as an environmental component were evaluated in conjunction with the dietary treatments.

Materials and Methods

The influence/interaction of dietary protein level and feeding rate on reproductive performance of female channel catfish crossed with male blue catfish to produce hybrid catfish fry was evaluated. Two diets containing 42 and 32% protein level were manufactured by ARKAT Feeds, Inc. (Dumas, AR, USA) using a commercial formulation for catfish. Two feeding frequencies (six times per week and three times per week), were used giving rise to four treatment combinations: 42/6–42% protein level, six feedings per week; 42/3–42% protein level, three feedings per week; 32/6–32% protein level, six feedings per week; 32/3–32% protein level, three feedings per week. Two channel catfish genetic groups (high and low spawning) (Ballenger 2007), each one with three ages (3, 4, and 5 yr old), were spawned during three periods (early, middle, and late season) (Table 1). Evaluation of the dietary protein level and feeding rate treatments were

TABLE 1. Number of channel catfish females (*Ictalurus punctatus*) classified by genetic group, age class, and period of spawning per dietary treatment.

Treatment	1	2	3	4	Total
Protein level	42%	42%	32%	32%	
Feed frequency	6	3	6	3	
Females by genetic group					
High spawning	44	55	44	44	187
Low spawning	55	57	56	59	227
Females by age class					
Three years old	33	35	30	37	135
Four years old	15	19	11	10	55
Five years old	51	58	59	56	224
Females by spawning period					
Early	30	28	29	29	116
Middle	13	15	13	14	55
Late	56	69	58	60	243

performed through spawning success, egg production (number of eggs per gram of egg mass [egg size] and number of eggs per gram of female body weight [fecundity]), and fertilization rate determined at 48 h after fertilization.

Experimental Fish

A total of 414 female channel catfish were maintained at the E. W. Shell Experimental Station, Auburn University. All brood stock were stocked in 16 ponds, using 4 ponds per treatment. The females were divided into two genetic groups on the basis of prior spawning behavior: high spawning (HS) and low spawning (LS); based on that characteristic they were assigned proportionally in a randomized manner to each pond (Table 1). Females were tagged with Passive Integrated Transponder (PIT) tags, to follow individual body weight, length, and girth. The fish were stocked in 0.04 ha ponds at a density of ~1130 kg/ha in February 2004. The acclimation period was approximately 1 mo, and the trial period was 70–90 d depending on the spawning period. Fish were fed during the warmest part of the day between 1500 and 1700 h, at a rate of 1.7% of total biomass of brood fish stocked per pond. Water quality parameters were taken

twice daily for dissolved oxygen and temperature and twice weekly for pH, ammonia-*N*, and nitrite-*N*.

For the first spawning period (early), two ponds of each treatment were drained, and 16 females (out of 32) were selected based on external characteristics (abdominal fullness, softness and palpability of the ovaries, redness or swollen appearance of the genitals). The second spawning period (middle) was performed using one pond of each treatment, and selecting 16 females from a total of 32. At the last spawning period (late) gravid females were selected from all remaining females in all ponds.

Females selected for induced spawning were transferred to holding tanks ($3.0 \times 0.47 \times 0.61$ m with a water volume of 670–837 L) supplied with continuous flow-through water, and placed individually in soft mesh bags. Total length, body weight, and girth were recorded. GMP grade luteinizing hormone releasing hormone analog (LHRHa) from American Peptide (Vista, CA, USA) was utilized to induce ovulation. Hormone injections were administered in two doses, a priming injection of 20 µg/kg LHRHa, followed 12 h later by a resolving dose of 100 µg/kg.

Collection and Fertilization of Gametes

Beginning 24 h after the second injection, females were monitored for ovulation. Females releasing eggs were removed from the holding tank and anesthetized in 250 mg/L tricaine methanesulfonate (MS-222) (Argent Chemical Laboratories, Redmond, WA, USA) buffered with 250 mg/L sodium bicarbonate. Females were stripped and eggs were collected in metal pie pans slightly lubricated with vegetable shortening. Females not expressing eggs were examined every 2–4 h for ovulations. Attempts to strip gametes ceased when no females remained that had swollen abdomens in response to the hormone treatment, and when all females had been anesthetized once to check for ovulation. Eggs were weighed, number of eggs per gram of egg mass, number of eggs per gram of female body weight, and total number of eggs were estimated for each female.

The eggs and blue catfish sperm were gently swirled together, allowed to sit for 2–10 min until they formed a mass, then the egg masses were placed in a water hardening trough with continuous, flowing, aerated reservoir water for 15 min. Finally, they were transferred to an egg basket in a hatching trough and held until hatch. The troughs had an air supply and a paddle wheel which were turned on when the youngest egg mass in the trough was at least 3 h old. Eggs were treated with formalin (100 ppm) or copper sulfate (32 ppm) three times daily to prevent fungal growth.

Fertilization Rate

The egg masses were evaluated 48 h after addition of sperm to determine the percentage of developing eggs (fertilization rate). A sample of eggs was placed in a clear Petri dish and a light shown through the bottom to view the eggs and estimate the percent that was developing (Dunham et al. 1998).

Egg Measurements

Two samples of eggs were obtained from the first set of eggs during the stripping of each female. One of the samples was placed in the freezer (–80 C) for later biochemical analysis, and the other sample was preserved using formalin (5%). The latter was used to determine the egg weight, as well as the egg diameter. Egg weight was determined from the total number of eggs in known weights of samples. Egg diameter was measured for 78 individual females randomly chosen from treatments 42/3 and 32/3, between ages 3 and 5 yr old. A sub-sample consisting of 15 eggs from each of the 78 individuals was randomly chosen and photographed. The software Image Pro-Express v. 4.5.1.3 (Media cybernetics, Bethesda, MD, USA) was used to determine egg diameter measurements from preserved eggs.

Analytical Procedures

Analysis of diets was conducted to determine crude protein, crude lipids, energy content, and moisture content. Crude protein content of the

diets was analyzed by micro Kjeldahl method and the crude lipid by the ether extraction method (Soxtec Avanti 2055 Manual Extraction Unit, Foss Tecator, Höganäs, Sweden). Determination of protein, lipid, and fatty acids profile was performed for 78 individual females randomly chosen from treatments 42/3 and 32/3, between ages 3 and 5 yr old using two subsamples of each individual. The crude lipid of the egg samples were extracted by using the method of Folch et al. (1957). Fatty acid methyl esters (FAMES) were analyzed using a hydrogen flame ionization gas chromatograph (GC-17A Ver. 3, Columbia, MD, USA) equipped with capillary column (Omegawax 530, 30 m \times 0.53 mm \times 0.5 μ m film thickness; Supelco 2-4019, Sigma-Aldrich, Oslo, Norway), using helium as the carrier gas. Sample FAMES were identified and quantified by comparing peak retention times and area counts to those of serially diluted mixtures of reference standards PUFA-3, Supelco 37 Component FAME Mix, and GLC 90 (Supelco, Bellefonte, PA, USA). Nonadecanoic acid methyl ester (C19:0) (Sigma-Aldrich, Inc., St. Louis, MO, USA) served as the internal standard. The results of the individual fatty acids were expressed as relative percentage of total identified FAMES and as milligram per individual egg.

Free amino acids were determined as total ninhydrin positive substances (TNPS) using a colorimetric determination (Lee and Takahashi 1966). TNPS were reported as micromoles per gram of eggs and micromole per 100 eggs.

Statistical Analysis

Relative weight (W_r) has been used to determine condition of the fish, however Neumann and Murphy (1992) consider it as a robust predictor of fecundity rather than growth. In the present study this index was used to determine whether or not feeding conditions and/or the quality of the food had an effect on the female brood fish. The relative weight (W_r) was determined by the equation (Anderson and Neumann 1996):

$$W_r = (W/W_s) \times 100, \quad (1)$$

where W is the weight of an individual and W_s is a length-specific standard weight predicted by a weight-length regression constructed to represent the species. The form of the W_s equation for catfish is, (Brown et al. 1995):

$$\log_{10}(W_s) = -5.800 + 3.294(\log_{10}TL) \quad (2)$$

where TL is the total length for channel catfish that are 70 mm or longer.

Relative weight (W_r) values were determined at the beginning and at the end of the trial, as well as the difference between these two values for each genetic group, and classified by age class. Then, an ANOVA procedure was performed to detect differences because of protein levels, feed frequencies, or the interactions of these two.

Analysis of the spawning success was performed using exact logit analysis. The model was constructed to include the effect of treatments (protein and frequency) on spawning for each genetic group, using the age of fish and period of spawning as covariates (Quintero et al. 2007a). Number of eggs per gram of egg mass and number of eggs per gram of female body weight were evaluated using the zero-inflated negative binomial regression (ZINB). This model used protein level, feed frequency, and their interactions as the explanatory variables, and genetic group, age of fish, and period of spawning as covariates (Quintero et al. 2007b). ANOVA was performed for fertilization rate following an arcsin transformation and for egg diameter measurements to detect treatment differences because of protein levels, feed frequency, and/or their interaction for each age class by genetic group (HS and LS). Analysis of protein and lipid content on the egg were performed using the beta regression model proposed by Ferrari and Cribari-Neto (2004). Fatty acid composition for treatments 42/3 and 32/3 was analyzed using *t*-test in terms of relative percentage (original values were transformed by taking the arcsin of their square root). If the arcsin variances were not normally distributed, the Kruskal–Wallis non-parametric test was applied to the non-transformed data. Ratios of essential fatty acids (DHA : EPA,

ARA : DHA, ARA : EPA) were also analyzed using a *t*-test. Statistical procedures from SAS[®] version 9.1 (SAS Institute, Inc., Cary, NC, USA) were used.

Results

Water quality parameters are given in Table 2. No significant difference among the treatments was detected for any of these parameters, except nitrite in ponds for the treatment 42% protein level fed 6 times per week, which had significantly higher amounts of nitrite (0.009 mg/L vs. 0.004–0.005 mg/L). The observed levels were suitable for culture of this species. Aerators were used infrequently and when necessary to maintain adequate dissolved oxygen levels. Mean dissolved oxygen values were kept higher than 7.0 mg/L in the morning, and higher than 10.0 mg/L in the afternoon.

Condition of the fish was characterized as poor, moderate, and good according to relative weight. Three-year-old fish, either HS or LS, were stocked in good condition (range 120–130), and were harvested in the same range (110–130) (Tables 3 and 4). Four-year-old HS fish had the poorest condition (range 65–78), while fish of the same age in LS were in moderate condition (range 106–112); but at the end of the trial, all fish were in good condition (mean relative weight values were 117 and 143 for HS and LS, respectively). Five-year-old fish from both genetic groups were initially in moderate condition (relative weight between 80 and 92), and similar to 4-yr-old fish, they reached a good condition by the end of the experiment (relative weight values from 115 to 125). In general, fish fed from high feed frequencies (42/6 and 32/6) had better final condition (higher relative weight values) than fish from low feed frequencies (42/3 and 32/3), except for 4-yr-old fish which did not exhibit significant differences in relative weights for any of the treatments (Tables 3 and 4).

Spawning success was significantly affected by age of fish and period of spawning for both genetic groups (Table 5). The odds of spawning for HS were not affected by either

dietary protein level or feed frequency, but age of fish had a highly significant effect ($P < 0.0001$). Thus, 5-yr-old females had 8.4 times higher odds of spawning than 3-yr-old females and 2.9 times higher than 4-yr-old females. Spawning success of females from LS was affected significantly by the change in frequency of feeding, but not by the change in the dietary protein level. For this reason, the odds of spawning from fish fed either 42/3 or 32/3 were 1.9 times that of fish fed 42/6 or 32/6. There was also a significant effect of age, with older fish having higher odds of spawning. Five-yr-old females had 4.9 times and 2.2 times higher odds of spawning than 3- and 4-yr-olds, respectively. Finally, as mentioned above, period of spawning had a significant effect on spawning success, with higher odds of spawning at the beginning of the spawning season than in mid or late season (8.6 and 16.3 times higher, respectively).

Egg production, either as number of eggs per gram of egg mass or number of eggs per gram of female body weight, included a relatively high number of zeroes (Figs. 1, 2). The presence of those zeros was a consequence of the significant effect of fish age, period of spawning, and feed frequency on spawning success. Also, fish age was found to be the only variable that significantly affects the number of eggs per gram of egg mass ($P < 0.0001$), while spawning period (middle period) significantly affected the number of eggs per gram of female body weight (Quintero et al. 2007b).

The ZINB regression model predicted the mean values of the number of eggs per gram of egg mass for age 3, 4, and 5 females to be 63, 55, and 49, respectively, and 53, 52, and 51 for early, middle, and late spawning periods, respectively. When analyzing number of eggs per gram of female body weight, the predicted means for females of age 3, 4, and 5 were 8.2, 8.3, and 7.5, respectively. For the same response variable the mean values were 7.8 for early season, 8.2 for middle season, and 6.9 for late season periods of spawning.

The relationship between total number of eggs versus total weight, and total length are described for each genetic group (Figs. 3, 4).

TABLE 2. Water quality parameters from earthen ponds holding female channel catfish, *Ictalurus punctatus*, for the entire acclimation and trial period (mean ± SD).

Treatment	1	2	3	4
Protein level	42%	42%	32%	32%
Feed frequency	6	3	6	3
Parameters				
Dissolved oxygen (mg/L) AM	7.0 ± 0.4	7.3 ± 0.6	7.0 ± 0.6	7.1 ± 0.4
Dissolved oxygen (mg/L) PM	10.5 ± 1.9	10.5 ± 0.3	10.3 ± 0.3	11.4 ± 0.6
Temperature (C) AM	20.1 ± 0.2	20.3 ± 0.2	20.2 ± 0.2	20.0 ± 0.3
Temperature (C) PM	23.2 ± 0.2	23.4 ± 0.2	23.5 ± 0.1	23.3 ± 0.1
pH	8.0 ± 0.3	8.2 ± 0.2	8.0 ± 0.3	8.1 ± 0.3
Total Ammonia Nitrogen (mg/L)	0.16 ± 0.13	0.07 ± 0.03	0.06 ± 0.01	0.07 ± 0.04
NO ₂ (mg/L)	0.009 ± 0.002 ¹	0.005 ± 0.0005	0.004 ± 0.0004	0.005 ± 0.003

¹Significantly different *P*-value = 0.0056.

TABLE 3. Initial relative weight, final relative weight, and difference between relative weights of channel catfish females after being maintained in dietary treatments by age class for high spawning group.

Treatment	Initial <i>W_r</i>	Final <i>W_r</i>	Difference
Age 3			
1-42/6	124.6 ± 12.8	126.7 ± 10.6 ^a	2.0 ± 14.1
2-42/3	121.0 ± 11.1	113.2 ± 10.4 ^b	-7.8 ± 8.8
3-32/6	124.2 ± 8.8	125.5 ± 11.8 ^a	1.3 ± 13.0
4-32/3	126.2 ± 13.0	118.8 ± 12.2 ^{ab}	-7.3 ± 13.2
<i>P</i> -value	0.5419	0.0015	0.0213
Age 4			
1-42/6	65.3 ± 11.0	117.5 ± 9.4	52.2 ± 8.3
2-42/3	75.3 ± 19.8	118.5 ± 21.7	43.2 ± 16.9
3-32/6	68.4 ± 12.4	117.5 ± 10.2	49.2 ± 10.4
4-32/3	77.7 ± 17.2	112.8 ± 11.1	35.1 ± 12.4
<i>P</i> -value	0.3542	0.8743	0.0687
Age 5			
1-42/6	86.9 ± 7.8	120.2 ± 12.9 ^{ab}	33.3 ± 15.4 ^{ab}
2-42/3	90.4 ± 11.9	120.4 ± 11.5 ^{ab}	30.0 ± 14.4 ^{ab}
3-32/6	85.8 ± 11.2	125.8 ± 9.9 ^a	40.0 ± 12.9 ^a
4-32/3	91.5 ± 14.4	114.9 ± 9.0 ^b	23.4 ± 12.1 ^b
<i>P</i> -value	0.396	0.0458	0.0076

Values followed by the same letter are not different (*P* > 0.05, Tukey-Kramer method) within each column.

TABLE 4. Initial relative weight, final relative weight, and difference between relative weights of channel catfish females after being maintained in dietary treatments by age class for low spawning group.

Treatment	Initial <i>W_r</i>	Final <i>W_r</i>	Difference
Age 3			
1-42 / 6	130.1 ± 15.3	128.7 ± 10.3 ^a	-1.4 ± 13.2 ^{ab}
2-42 / 3	129.8 ± 16.5	118.8 ± 9.1 ^b	-10.9 ± 11.4 ^b
3-32 / 6	125.9 ± 11.8	129.3 ± 9.2 ^a	3.5 ± 13.4 ^a
4-32 / 3	129.5 ± 19.0	123.8 ± 10.6 ^{ab}	-5.6 ± 10.6 ^{ab}
<i>P</i> -value	0.8797	0.0175	0.0172
Age 4			
1-42 / 6	108.8 ± 38.0	140.3 ± 61.8	31.5 ± 24.7
2-42 / 3	111.6 ± 26.6	150.0 ± 41.3	38.3 ± 14.7
3-32 / 6	106.0 ± 19.2	130.8 ± 29.8	24.8 ± 11.4
4-32 / 3	117.0 ± 59.5	168.1 ± 67.7	51.2 ± 8.2
<i>P</i> -value	0.9862	0.8681	0.4766
Age 5			
1-42 / 6	85.9 ± 10.9	123.7 ± 12.8 ^a	37.8 ± 13.8 ^{ab}
2-42 / 3	84.4 ± 13.5	115.9 ± 10.6 ^b	31.5 ± 13.8 ^b
3-32 / 6	80.8 ± 11.6	122.6 ± 12.5 ^a	41.8 ± 15.6 ^a
4-32 / 3	83.4 ± 11.3	115.4 ± 10.8 ^b	32.1 ± 10.1 ^b
<i>P</i> -value	0.3154	0.0019	0.0019

Values followed by the same letter are not different (*P* > 0.05, Tukey-Kramer test) within each column.

Variation in total female body weight explained 55 and 40% of the variability in the total number of eggs for HS and LS, respectively. The percentage variability in total number of eggs that was explained by length was 48% for HS, 1 and 26% for LS. Variation in biomass gained (lost) explained 24 and 3% of the variability in the total number of eggs for HS and LS, respectively.

Fertilization rates from eggs produced by females of the HS were in the range of 42–90%, with an average of 67.5% (CV = 24.9%). Fertilization rates from females of the LS were in the range of 36–95%, with a mean value equal to 64.4% (CV = 29.6%). There was not significant difference among fertilization rates from treatment combinations in each one of the genetic groups. The 42/6 treatment had the

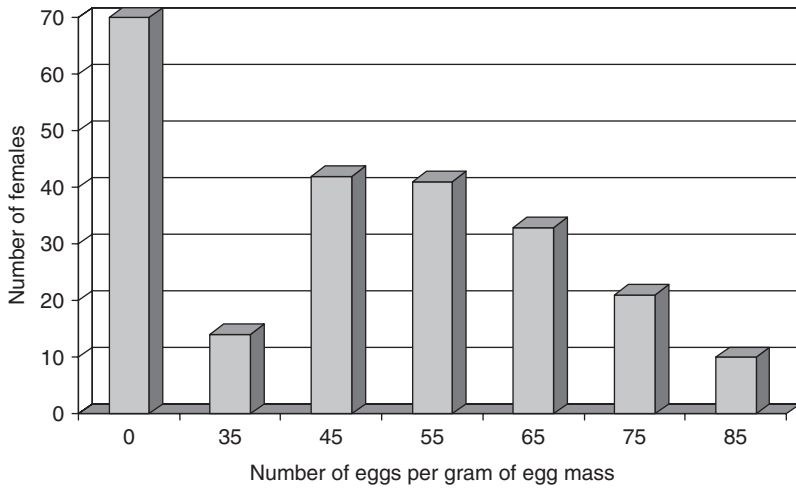


FIGURE 1. Distribution of number of channel catfish females, *Ictalurus punctatus*, versus number of eggs per gram of egg mass.

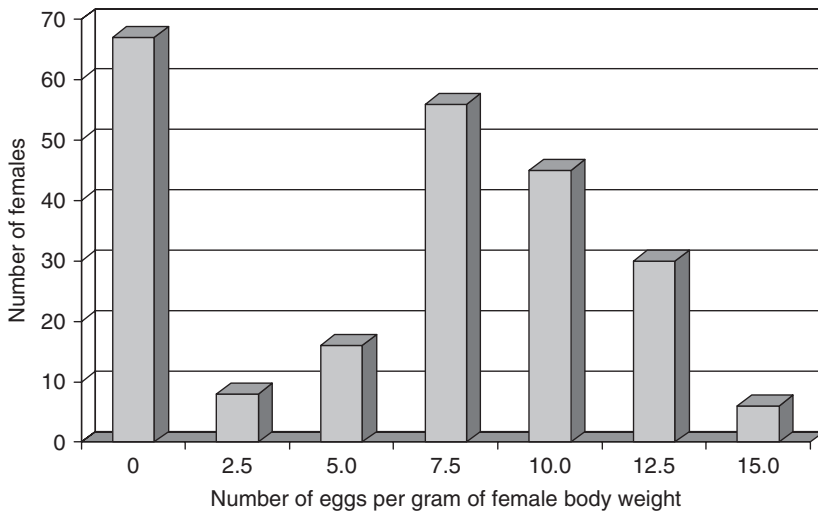


FIGURE 2. Distribution of number of channel catfish females, *Ictalurus punctatus*, versus number of eggs per gram of female body weight.

highest observed mean for both genetic groups. Six day per week feeding appeared to benefit the HS and high protein appeared to benefit the LS.

Egg diameters from HS females averaged 3.35 mm (CV = 9.11%), and those from LS females averaged 3.36 mm (CV = 7.64%). Egg diameters from 3-yr-old females were significantly different from 5-yr-old females ($P < 0.0001$) with mean values of 3.15 and

3.45 mm, respectively. Considering egg diameters for 3-yr-old females, there was a significant difference between genetic groups (HS–3.11 mm, LS–3.20 mm), as well as for 5-yr-old females (HS–3.46 mm, LS–3.42 mm), with P -values equal to 0.0001, and 0.0153, respectively. Differences among diet treatments for each genetic group were detected when data were compartmentalized as a function of female age (3- and 5-yr-old) (Table 6).

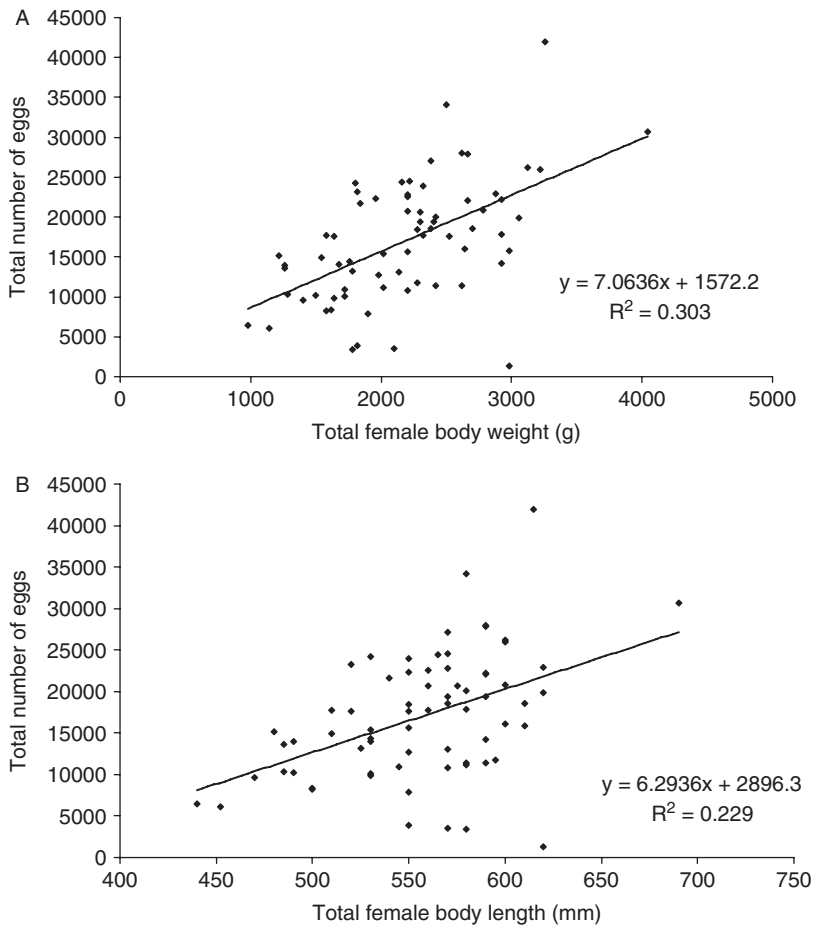


FIGURE 3. Total number of eggs produced by channel catfish females (high spawning), *Ictalurus punctatus*, versus A total female body weight and B total female body length.

Biochemical analysis from feed and channel catfish eggs produced by dietary treatments are displayed in Table 7. Moisture, protein, lipid, and energy content from the feed were determined for dietary treatments. The 42% protein diet had significantly higher values for each of the variables analyzed than those reared on the 32% protein diet (Table 7). Egg composition and relative percentages of protein and lipids were not significantly different among treatments, but free amino acids were significantly higher for 42% protein diet. Egg fatty acid composition is displayed in Tables 7 and 8. The most abundant fatty acids were 16:0, 18:0, 18:1n9, 18:2n6, 20:3n3, and 22:6n3. There was a significant effect of protein level on the fatty

acid composition, except for the following fatty acids: 14:0, 16:0, 18:2n6, and 20:1n9. Proportions and absolute values of linolenic acid and highly unsaturated fatty acids (ARA, EPA, and DHA), as well as the *n3:n6* ratio were significantly higher in eggs from fish fed 42% protein diet (Tables 7, 8). The ratios DHA : EPA, ARA : EPA, and ARA : DHA were significantly higher in eggs from fish fed 32% protein diet (Table 7).

Discussion

Feeding activity of channel catfish roughly paralleled water temperature, decreasing in November to the lowest levels in December

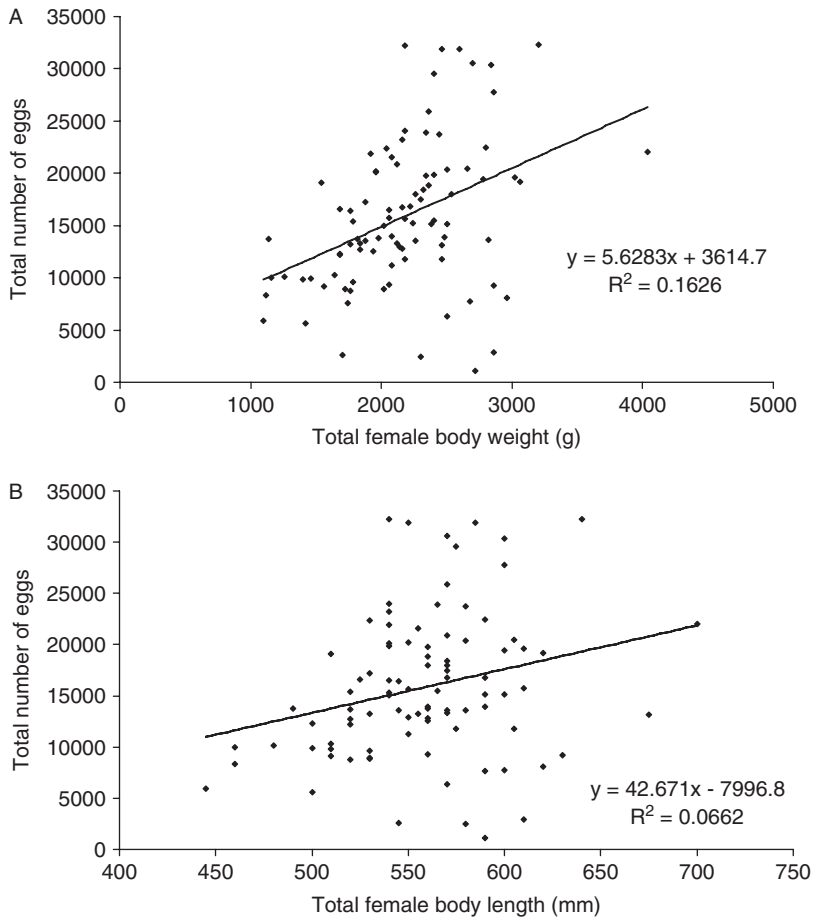


FIGURE 4. Total number of eggs produced by channel catfish females (low spawning), *Ictalurus punctatus*, versus A total female body weight and B total female body length.

through February, and increasing from March to June, reaching highest levels in July and August (MacKenzie et al. 1989). Our observations were limited to late winter (February) and the spring season (March to June), and they followed this same pattern, a very low response during February, and then increased feeding activity during the spring. These observations translated into growth responses and increased condition index (relative weight), which was strongly marked in 4- and 5-yr-old catfish, but very poor or even negatively affected in the 3-yr-old fish. Communal stocking/evaluation may have contributed to this unexpected result with 3-yr-old females not being competitive

with 4- and 5-yr-old females. Previously, Dunham et al. (1982) validated communal evaluation for growth experiments with fingerling and food-fish sized ictalurid catfish. Our results indicate that the effects of communal evaluation may be different for older, brood fish sized catfish, and this technique should be re-evaluated for brood fish experiments.

One of the reported benefits from protein contribution is an earlier maturation of gonads and eggs in larger brood stock (El-Sayed et al. 2003; Chong et al. 2004). This was not the case in channel catfish, because spawning success and fecundity parameters were related to fish age rather than fish size. Effects from variation in protein in terms of fecundity or fertilization

TABLE 5. Results of exact logistic regression analysis evaluating differences in spawning success (ovulation rate) from channel catfish females, among two dietary protein levels, and two feed frequencies, for two genetic groups (high and low spawning) using age of fish and period of spawning as covariates.

Variable	Parameter			Odds ratio
	DF	Parameter	$P > \chi^2$	
High spawning				
Protein	1	0.1215	0.1215	
Feed frequency	1	0.8619	0.8619	
Protein × feed frequency	1	0.1184	0.1184	
Age	1	<0.0001	<0.0001	2.8
Period 1*	1	0.0022	0.0022	8.6
Period 2*	1	0.9533	0.9533	
Low spawning				
Protein	1	-0.1751	0.2707	
Feed frequency	1	0.315	0.0427	1.9
Protein × feed frequency	1	0.1213	0.4312	
Age	1	0.7988	<0.0001	2.2
Period 1 ^a	1	1.3963	<0.0001	16.3
Period 2 ^a	1	-0.2734	0.7776	

^aPeriod 1 and period 2 correspond to the logistic regression coefficients where the levels of period are coded as period 1 (1, 0), period 2 (0, 1) and period 3 (-1, 1).

TABLE 6. Egg diameter (millimeter) measurements (mean ± SD) from channel catfish females, *Ictalurus punctatus*, related to dietary treatments by genetic group (high and low spawning) and age class.

Treatment combination	Age 3	Age 5
High spawning		
1-42/6	3.21 ± 0.14 ^a	3.57 ± 0.31 ^a
2-42/3	3.15 ± 0.21 ^a	3.53 ± 0.24 ^{ab}
3-32/6	3.15 ± 0.09 ^a	3.44 ± 0.20 ^b
4-32/3	2.94 ± 0.21 ^b	3.28 ± 0.34 ^c
<i>P</i> -value	<0.0001	<0.0001
Low spawning (strain 2)		
1-42/6	3.22 ± 0.17	3.36 ± 0.26 ^b
2-42/3	3.15 ± 0.20	3.47 ± 0.19 ^a
3-32/6	3.23 ± 0.09	3.52 ± 0.29 ^a
4-32/3	3.19 ± 0.23	3.31 ± 0.22 ^b
<i>P</i> -value	0.3438	<0.0001

Values followed by the same letter are not different ($P > 0.05$ Tukey-Kramer test) within each column.

rate (48 h) were not significant, but there was an effect on egg size. Thus, females given higher protein diets and fed more frequently tended to

have larger eggs. Fertilization rate determined at 48 h was not significantly different among treatments for each genetic group ($P = 0.0606$ for HS, and $P = 0.1480$ for LS) with values ranging from 61.0 to 74.2%, and may be considered in the normal range from previous studies, where 70–80% fertilization rates has been achieved (Dunham et al. 1998). Additionally, a genotype (genetic group) × environment (feeding frequency) interaction was found for spawning success probabilities, but not for differing levels of protein (Table 5). Biochemical composition of channel catfish eggs in terms of protein and lipid content was not affected by the dietary treatment. However, the free amino acid content was significantly higher in eggs from females fed with the higher protein diet. Fatty acid composition of channel catfish eggs from females fed with 42% protein diet had significantly higher relative values and absolute values (milligrams per 100 eggs) than those from females fed with 32% protein diet, exception to this trend were the following fatty acids: 14:0, 16:0, 18:2n6, and 20:1n9. The effect of these higher levels of fatty acids and free amino acids was not evaluated in subsequent stages of the egg development.

The usefulness of relative weight (W_r) as a condition index for determining the quantity and quality of the eggs was not evident for the females of channel catfish spawned during this study. Although condition of the fish was affected by dietary treatments, the relative weight values did not exhibit a clear relationship with the egg mass or the number of eggs per gram of female body weight obtained from spawned females. The best and worst spawning age classes had similar relative weight values.

Age of the fish was found to significantly affect the probability of spawning for both genetic groups, with older fish having higher probability of successful spawning than younger fish. Several authors have pointed out this effect. Santiago (1979) reported a very low spawning success in channel catfish 3-yr-old females (12.7%). Dunham et al. (1983) found that 4-yr-old fish of the Kansas strain had a greatly improved spawning rate compared to when they were 3 yr old, suggesting that

TABLE 7. Proximate analysis from commercial channel catfish feed and biochemical composition from eggs of channel catfish females, *Ictalurus punctatus*, including proteins, lipids, free amino acids as TNPS, essential fatty acids, and ratios between essential fatty acids from dietary treatments 42 and 32% protein level offered three times per week.

Parameter	42/3	32/3	P-values
Feed			
Moisture (%)	9.41 ± 0.09	7.12 ± 0.19	0.0048
Protein (%)	40.74 ± 0.83	33.99 ± 1.03	0.0187
Lipids (%)	6.22 ± 0.05	5.48 ± 0.11	0.0137
Energy (cal)	4437 ± 6	4231 ± 17	0.0036
Eggs			
Protein (%)	17.80 ± 1.93	17.21 ± 1.71	0.1590 ^a
Protein (mg per individual egg)	3.20 ± 0.98	2.95 ± 0.94	0.1531
Lipids (%)	6.82 ± 1.05	6.49 ± 1.05	0.6159 ^a
Lipids (mg per individual egg)	1.12 ± 0.42	1.02 ± 0.39	0.1415
TNPS (μmol/g egg mass)	3.84 ± 1.89	2.43 ± 2.05	<0.0001
TNPS (μmol per individual egg)	6.91 ± 3.60	3.74 ± 3.09	<0.0001
Fatty acids—absolute values (mg per 100 eggs)			
Linoleic acid (18:2n6)	9.18 ± 3.53	8.08 ± 3.01	0.0933
Linolenic acid (18:3n3)	0.53 ± 0.22	0.31 ± 0.13	<0.0001
ARA (20:4n6)	0.11 ± 0.05	0.07 ± 0.03	<0.0001
EPA (20:5n3)	1.60 ± 0.76	0.36 ± 0.23	<0.0001
DHA (22:6n3)	9.41 ± 3.70	5.13 ± 2.27	<0.0001
Ratios			
DHA : EPA	6.46 ± 1.98	13.27 ± 3.09	0.0001
ARA : DHA	0.012 ± 0.005	0.015 ± 0.003	0.0004
ARA : EPA	0.08 ± 0.07	0.19 ± 0.07	<0.0001

TNPS = total ninhydrin positive substances; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

^aThe smallest P-value 0.05 from beta regression coefficients.

they had just reached sexual maturity. Moore (1986) showed that for older fish, as fish weight increased percent success of spawning and hatch percentage generally increased, and a similar trend was observed in younger fish, but data for the younger fish were not conclusive. Davis et al. (2005) also suggested that age rather than size is a more important component of maturation. These authors proposed to expose fish to shortened seasonal changes that mimic increased age combined with intensive feeding of fish, as a solution to overcome age as a limiting factor in brood stock performance (Davis et al. 2005).

The spawning period had a significant effect on the estimated odds of successful spawning in both genetic groups, with higher probabilities of spawning during the early season. Broussard and Stickney (1981) evaluated four channel catfish strains (Minnesota, Uvalde, Auburn, and

Rio Grande strains) and found distinctive patterns in spawning periods for each strain. Similar results were found by Dunham et al. (1983) who evaluated crossbreed and pure-strain mating from channel catfish derived from Kansas, Marion, Auburn, and Rio Grande strains.

Fecundity is a trait that is a function of the size or age of the fish. According to some authors, the ability to reproduce does not depend on age but rather on the attainment of an adequate body size (Sokolowska and Skóra 2001). Thus, most analyses of fecundity have primarily considered female body weight (Froese and Luna 2004). However, relative fecundity in channel catfish studies has been poorly correlated with female body weight (Bice 1981; Walser and Phelps 1993; Argue 1996; Lambert 1998). For instance, Brauhn and McCraren (1975) found that pre-spawning gonadosomatic indices ($GSI = \text{gonad}$

TABLE 8. Fatty acid analysis from eggs of channel catfish females, *Ictalurus punctatus*, by dietary treatments, commercial catfish diet 42 and 32% protein level offered three times per week.

Fatty acid	42/3	32/3	P-value
14:0	0.99 ± 0.01	0.90 ± 0.04	0.0961
16:0	18.78 ± 0.02	18.37 ± 0.03	0.0702
16:1n7	3.08 ± 0.02 ^a	2.37 ± 0.04 ^b	<0.0001
18:0	12.17 ± 0.02	13.10 ± 0.02	<0.0001
18:1n9	28.67 ± 0.11	30.70 ± 0.05	0.0001
18:2n6	7.25 ± 0.07	6.97 ± 0.04	0.2471
18:3n6	ND	ND	ND
19:0	5.92 ± 0.04	5.89 ± 0.04	0.8472
18:3n3	0.41 ± 0.01	0.26 ± 0.002	<0.0001
20:1n9	1.08 ± 0.01	1.13 ± 0.01	0.1607
20:2n6	1.16 ± 0.004	1.42 ± 0.01	<0.0001
20:3n6	2.65 ± 0.02	3.25 ± 0.01	<0.0001
20:3n3	3.88 ± 0.04	5.72 ± 0.01	<0.0001
20:4n6	0.08 ± 0.002	0.06 ± 0.001	<0.0001
20:5n3	1.20 ± 0.03	0.35 ± 0.01	<0.0001
22:4n6	0.24 ± 0.003	0.37 ± 0.002	<0.0001
22:5n6	1.11 ± 0.02	2.56 ± 0.02	<0.0001
22:5n3	0.74 ± 0.004	0.55 ± 0.01	<0.0001
22:6n3	7.34 ± 0.03	4.38 ± 0.08	<0.0001
Σn-6	11.37 ± 0.08	13.10 ± 0.05	<0.0001
Σn-3	13.66 ± 0.02	11.31 ± 0.07	<0.0001
n-3 / n-6	1.22 ± 0.18	0.84 ± 0.12	<0.0001

weight/body weight × 100) were not uniform among size classes. Ovary weights were not increasing proportionally with increasing fish size. Thus, female body weight used in the current study ranged from 0.4 to 3.4 kg for HS and 0.6 to 3.1 kg for LS, with a combined mean weight of 1.74 kg. The observed weight discriminated by age revealed overlapping of bodyweights. For instance, the range for 3-yr-old females was 1.1–3.4 kg, 4-yr-old females was 0.4–2.2 kg, and 5-yr-old females was 0.6–3.1 kg. Absolute fecundity was strongly correlated with body weight in the HS and moderately correlated in the LS (Figs. 3A, 4A). These factors explained the poor correlation observed between total number of eggs and total weight, biomass gain, and/or total length of the female (Figs. 3, 4).

The effect of age on fecundity and egg size indices of freshwater fishes remains underexplored in contrast to marine species (Shatunovskii 2006). In channel catfish aquaculture, female age has been found responsible

for spawning performance rather than its size (Santiago 1979; Dunham et al. 1983a). Brauhn and McCraren (1975) observed that the pre-spawning GSI by age class did not show any trend, however low was their sample size (three individuals for classes 3 to 6 yr old). In the present study, results from the ZINB analysis found that number of eggs per gram of egg mass had a significant effect of age on this trait, older females had larger eggs. According to our figures they did not have a lower number of eggs. According to Shatunovskii (2006), this phenomenon can be attributed to an increased reproductive function in ontogeny, which is realized as a more active synthesis of ovovitellin in the liver and its storage in oocytes as well as to an elongated period of trophoplasmatic growth of oocytes. This result has also been observed in walleye, where female age accounted for a greater amount of variation in egg mass than fork length or size (Johnston 1997).

A significant relationship exists between egg diameter and female size in some species of fish (Mann and Mills 1985; Wright and Shoesmith 1988; Bromage 1995). However, in other cases, egg size did not increase with parental size (Kamler 1992). Abdoli et al. (2005) suggested that female body size per se does not directly affect egg size, but rather the apparent effect of body size may result from age differences. Markmann and Doroshov (1983) monitored ovarian maturation in channel catfish finding a highly significant correlation ($r = 0.903$, $P < 0.001$) between mean oocyte diameter and GSI, which is an indicator of ovarian maturation. Similar results were described for the maturation cycle, where GSI was found to be low after spawning, increased from September to January, did not change in January to March, and a profound increase occurred to maximum values in April (MacKenzie et al. 1989). However, channel catfish female weight has been negatively correlated with average egg weight (Broussard and Stickney 1981). In the present study, there was no significant relation between egg size and either fish weight or fish length, but

there was a significant difference in egg diameter because of fish age (older females had bigger eggs).

Conclusion

Female brood stock increased their body weight by the end of the trial period (except for females 3 yr old, fed three times per week), and that response was significantly different according to the dietary treatment. In terms of spawning response, changing the protein level of the diet from 32 to 42% or increasing the feeding frequency from 3 to 6 times per week did not influence spawning rate. Additionally, older fish performed better than younger and the early spawning period was better than the later spawning period, regardless of the genetic group, for spawning rate. Number of eggs per gram of egg mass, and number of eggs per gram of female body weight were significantly affected by period of spawning and female age. Fertilization rate at 48 h was not significantly affected by protein level or feed frequency in any of the genetic groups; however, the dietary treatment affected egg size. Biochemical composition of channel catfish eggs in terms of free amino acids and fatty acid composition was affected significantly by the dietary treatment. The impacts of these egg quality changes on subsequent embryonic development and viability, and fry performance need to be evaluated.

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