



# Influence of forage fish and dietary lipid supplements on egg quality and fry production in channel catfish (*Ictalurus punctatus*) × blue catfish (*Ictalurus furcatus*) hybridization

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## Abstract

Hybrid catfish (channel catfish *Ictalurus punctatus* × blue catfish *Ictalurus furcatus*) display characteristics that are favourable to aquaculture production. Low hatch percentages are a principal reason this hybrid is not used widely in the catfish industry. This study was conducted to determine whether additional food source rich in lipids may lead to a higher quality egg production. A 10-week feed trial was conducted in ponds in Auburn, AL. A total of 219 female Kansas Select channel catfish were stocked into nine ponds, 0.04 ha in size. Three dietary treatments were randomly allocated to the ponds. Diet-1 was a standard 60 g kg<sup>-1</sup> lipid floating catfish feed. Diet-2 was the same feed supplemented with forage fish at ~28 kg ha<sup>-1</sup>. The third diet was the aforementioned catfish feed topcoated with 20 g kg<sup>-1</sup> lipid [10 g kg<sup>-1</sup> menhaden fish oil, 5 g kg<sup>-1</sup> high docosahexaenoic acid (DHA) oil and 5 g kg<sup>-1</sup> high arachidonic acid oil]. Results indicate that brood fish fed the high lipid diet spawned larger egg masses and had larger eggs both in weight and in diameter, with increased complements of fatty acids such as DHA, eicosapentaenoic acid and total n-3 fatty acids. The neutral and polar lipid fractions are also presented.

**KEY WORDS:** brood stock nutrition, channel catfish, fatty acids, highly unsaturated fatty acid, hybrid catfish, lipids

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## Introduction

The channel catfish aquaculture is an industry in the United States dating back to the 1930s. Production has increased to

a total of ~263 000 metric tons in 2006 worth an estimated \$481 million (USDA 2007). In recent years, the foreign production and importation of catfish fillets have increased to substantial levels. In the 3-year period between 1997 and 2000, catfish fillets imported into the USA increased eightfold from ~430 to ~3700 metric tons. The introduction of these imported products and loss of market share have negatively impacted the farm-gate price for American producers (Quagraine & Engle 2002).

With the interest of increasing the efficiency of production of catfish aquaculture operations, channel catfish (*Ictalurus punctatus*) females have been crossed with blue catfish (*Ictalurus furcatus*) males to produce a hybrid. Research on this hybrid catfish has shown that it has many promising characteristics such as increased growth, resistance to disease, carcass yield and a greater ease of seining (Ella 1984; Dunham & Argue 1998, 2000; Argue *et al.* 2003). The natural hybridization of these species is sporadic, however, and the hybrid vigour is witnessed almost exclusively in the F1 generation (Dunham & Argue 2000). The large scale production of this hybrid relies upon the techniques for hormone induction and strip spawning of the channel catfish female and artificial fertilization with blue catfish sperm (Sneed & Clemens 1959; Busch 1985; Dunham 1993; Dunham *et al.* 1999). The principal factor limiting this hybrid from being incorporated significantly into the industry is chronically low egg survival in the hatchery (Dunham & Smitherman 1987; Dunham *et al.* 1999). The survival rates for these hybrid embryos are typically half that of a straight channel × channel cross (Dunham & Argue 2000). Dunham *et al.* (1999) conducted experiments with different strains of channel catfish and fertilization techniques and concluded that, if fertilized correctly, the percentage of eggs that are fertilized for the hybrid crosses is not significantly different from that of pure channel catfish. When the same eggs were carried

through to hatch however, those that were hybridized hatched out at a significantly reduced proportion. It can be hypothesized then, that the reduced hatch percentage witnessed in the hybrid strain is probably associated with embryonic development within the egg after fertilization. In this phase of rapid development and differentiation, the embryo must get its nutrients, metabolic energy and structural components solely from reserves within the egg. The egg, then, should provide the developing embryo with a complete 'start up package' of DNA, RNA, enzymes, hormones, ions, proteins, lipids and combinations of all of the above (Wiegand 1996; Brooks *et al.* 1997). The egg profile, the quantity and quality of these components, largely depends upon the female parent and her diet (Brooks *et al.* 1997; Izquierdo *et al.* 2001).

While many of these components can be produced by the parent, some cannot be synthesized *de novo*. These elements, such as essential amino and fatty acids, must come from the diet (Brooks *et al.* 1997; Izquierdo *et al.* 2001). Highly unsaturated fatty acids (HUFAs) are both vital components in egg quality and ingredients that are frequently limiting in brood stock diets (Watanabe 1993; Fernandez-Palacios *et al.* 1995; Sargent 1995; Navas *et al.* 1997; Sargent *et al.* 1999; Furuita *et al.* 2000). The negative effect of HUFA deficient diets on brooder performance is well documented in marine species, but has gone relatively overlooked in freshwater genera. Marine fish have little or no ability to create HUFAs from lipid molecule precursors while many freshwater fish, including catfish, do display such an ability. This is one reason that diets rich in polyunsaturated fatty acids (PUFAs) such as linoleic (18:2n6) and linolenic (18:3n3) acids are effectively used for the growout of channel catfish. The biosynthetic production of HUFAs from precursors seems sufficient to satisfy the growth rate of this stage of their life (Gatlin & Stickney 1982).

Female brood fish, that are diverting a considerable portion of energy and nutrition to their developing oocytes, may have different dietary requirements to facilitate the development of these tissues (Sargent 1995; Wiegand 1996; Brooks *et al.* 1997). Paramount among these requirements is typically a high quality lipid source. Sargent *et al.* (1999) suggests that of all the PUFAs in the diets of brood fish, the three most important (and frequently limiting) are the HUFAs arachidonic acid (20:4n6) (ARA), eicosapentaenoic acid (20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA). Although able to endogenously supply their own HUFAs, female channel catfish may have a limitation to this ability and it may fall short of the optimum supply for incorpora-

tion into the egg. If such a limitation is present, increasing the incorporation of these HUFAs into the egg beyond the metabolic capacity requires dietary supplementation of the female brood fish. Consequently, the objective of this study was to determine whether increased long-chain lipid intake, through dietary supplementation or suitable prey items, leads to increased production of high quality eggs in female channel catfish and thus increased per cent hatch of their hybrid offspring.

## Materials and methods

### *Brood stock and feeding*

A 10-week feed trial was conducted in ponds at the E.W. Shell Fisheries Center in Auburn, Alabama. In March, two-hundred and nineteen 4-year-old female 'Kansas select' channel catfish brood stock were stocked into nine ponds, 0.04 ha in size, for an approximate stocking rate of 1332 kg ha<sup>-1</sup> per pond. Three dietary treatments were developed and randomly assigned to ponds, allowing for three replicates per treatment with 24–25 fish per pond. Treatment 1 was a standard 320 g kg<sup>-1</sup> crude protein, ~60 g kg<sup>-1</sup> lipid floating catfish feed. Treatment 2 was the same feed supplemented with forage fish (bluegill *Lepomis macrochirus* and fathead minnows *Pimephales promelas*) at ~28 kg ha<sup>-1</sup>, each. Treatment 3 was the standard catfish feed topcoated with 20 g kg<sup>-1</sup> lipid (10 g kg<sup>-1</sup> menhaden fish oil, 5 g kg<sup>-1</sup> high DHA oil and 5 g kg<sup>-1</sup> high ARA oil). The DHA and ARA oils (DSM Nutritional Products, Kaiseraugst, Switzerland) contained ~40% of the designated HUFA. Fatty acid analyses of the feeds that were used are presented in Table 1. The standard and topcoated diets contain 94, 104, moisture; 329, 322 protein, 59.5, 75.8 fat, 50.6, 46.4 fibre and 70.5, 67.4 mg kg<sup>-1</sup> ash respectively. The fish were fed the prepared feeds three times a week at an approximated 1.5% body weight per feeding. Dissolved oxygen (DO) and water temperature were measured twice daily at dawn and dusk and low DO events were mitigated by nighttime aeration. Total -ammonia-N, -nitrite-N and pH parameters were measured twice a week. No significant difference among the treatments was detected for any of these parameters, except total-ammonia-N in ponds that had topcoated feed, which had significantly higher amounts of total ammonia (0.36 versus 0.18–0.28 mg L<sup>-1</sup>). The observed levels were suitable for culture of this species. Mean DO values were kept higher than 6.5 mg L<sup>-1</sup> in the morning, and higher than 8.0 mg L<sup>-1</sup> in the afternoon.

**Table 1** Fatty acid profiles of two test diets used to examine different lipid amounts and sources and their affect on egg quality of channel catfish. Diet one was a standard commercial catfish diet (SD) and the second was the same feed topcoated 2% with HUFAs (TC)<sup>1</sup>

Fatty acid	Standard diet (SD) Relative %	Topcoated (TD) Relative %
14:0	1.36	3.27
14:1	0.07	0.10
15:0	0.17	0.29
15:1	0.10	0.08
16:0	17.90	18.24
16:1n7	2.48	3.78
16:2	0.15	0.34
16:3	0.15	0.48
16:4	ND	0.15
17:0	0.31	0.37
18:0	4.87	4.94
18:1n9	30.25	22.26
18:1n7	1.81	1.84
18:2n6	32.47	21.34
18:2n4	ND	0.08
18:3n6	0.29	0.26
18:3n3	2.18	1.62
18:4n3	0.17	0.56
20:0	0.29	0.33
20:1n9	0.87	0.84
20:2n6	0.31	0.30
20:3n6	0.23	0.62
20:4n6	0.27	4.88
20:4n3	0.10	0.41
20:5n3	0.96	2.98
22:0	0.17	0.34
22:1n9	0.15	0.07
21:5n3	ND	0.11
22:4n6	ND	0.09
22:5n6	ND	1.46
22:5n3	0.25	0.66
22:6n3	0.77	5.65
24:0	0.14	0.31
n/a	0.77	0.79
Total % n3	4.42	11.99
Total % n6	33.57	28.95
Ratio: n3/n6	0.132	0.414

ND, components that were below detectable limits.

<sup>1</sup> Analysis by New Jersey Feed Laboratory, Inc., Trenton, NJ, USA.

### Spawning and incubation

Hormone injection, strip spawning and egg incubation techniques were adapted from Jensen *et al.* (1983), Dunham (1993) and Campbell (1999). When water temperatures increased to 23–25 °C in May, the females were harvested from the ponds for spawning. Those exhibiting good spawning characteristics such as distended abdomens and swollen papillae were selected for hormone injection to initiate ovulation. Total length and body weight were recorded and used to calculate relative weight ( $W_r$ ). This condition index is determined by the equation:

$$W_r = (W/W_s) \times 100 \text{ (Anderson \& Neumann 1996),}$$

where  $W$  is the weight, in grams, and  $W_s$  is a length-specific standard weight obtained from a weight-length regression for channel catfish. The equation for  $W_s$  in catfish is:

$$\log_{10}(W_s) = -5.800 + 3.294 (\log_{10} \text{ TL}) \text{ (Brown } et al. 1995),$$

where TL is the total length of the fish. The fish were then transferred individually into soft mesh bags, and then into holding tanks (per treatment) that were supplied with continuous flow-through water. The holding tanks were 3.0 × 0.47 × 0.61 m in size containing 670–837 L of water.

Hormone injections were administered in two doses; a priming injection of 30 µg kg<sup>-1</sup> luteinizing hormone releasing hormone analogue, followed 12 h later by a resolving dose of 150 µg kg<sup>-1</sup>. Hormone was purchased from American Peptide (Sunnyvale, CA, USA). Forty hours after the first injection, females were monitored for ovulation. Females with soft abdomens and ovulated eggs adhering to their mesh bag were removed from holding tanks and anaesthetized in buffered 150 mg L<sup>-1</sup> tricaine methane sulphonate (MS-222) (Argent Chemical Laboratories, Redmond, WA, USA). Females were then stripped and eggs were collected, in 50–150 g quantities, in metal pans previously lubricated with vegetable shortening. Those females that did not express eggs were returned and rechecked later. Stripping of gametes ceased when all females had been stripped or attempts to strip them had been made.

Eggs were collected for later biochemical and visual analyses; the remaining mass was quantified gravimetrically and fertilized with blue catfish sperm at ~6.5 × 10<sup>7</sup> per 100 g of eggs. To obtain the sperm, the males were killed, their testes removed, cleaned and then macerated in a saline solution. This sperm-fluid homogenate was then filtered for solid tissue masses and blood contamination was kept to a minimum. The sperm was evaluated in terms of quantity and quality to minimize the adverse effects on hybridization. Then, it was diluted and refrigerated prior to stripping of the eggs and fertilization. Saline solution was then added to the eggs and sperm and gently mixed. Fresh water was then added to the pan to activate the gametes and the egg mass was allowed to water harden for 10–60 min. When the egg had formed a cohesive mass, they were transferred to an egg basket that was placed with eight other baskets in a 154 L trough with 6 L min<sup>-1</sup> water exchange, two air stones and a slowly turning paddle wheel. All eggs within a trough were from the same treatment. The water, on flow through from an adjacent pond, had ammonia levels below 0.2 ppm and nitrite levels below 0.02 ppm. Oxygen was spot checked in the troughs to assure that DO was above 5.0 ppm at all times.

Pond temperatures during this time averaged 25–26 °C in the morning and 28–29 °C in the evening.

Paddle wheels were turned on after the youngest egg was at least 4 h old. Formalin treatments of 100 mg L<sup>-1</sup> began 12 h after fertilization. After 24 h, the eggs received three treatments per day alternating between 35 mg L<sup>-1</sup> copper sulphate and 100 mg L<sup>-1</sup> formalin. Just prior to hatch, copper treatments were withheld and the formalin treatment was reduced to 50 mg L<sup>-1</sup> due to the increased vulnerability of embryos at this stage (Small & Chatakondi 2006). Individual egg masses were weighed and visually assessed 24 h pre-hatch to determine per cent viable fry. Once the eggs had hatched and sac fry had fallen to the bottom of the trough, they were enumerated by gravimetric techniques.

### Egg analysis

After spawning, the eggs that were collected before fertilization and water hardening were assessed by average weight and diameter as well as biochemical composition. Eggs were collected in two samples; one sample (~7 mL) that was frozen at -70 °C for later biochemical analysis and a second (~30 eggs) that was stored in preweighed vials containing 5 mL of a 5% formalin solution. The total weight of the vial and eggs ( $\pm 0.01$  g) was recorded. The formalin-preserved sample was used to assess weight and diameter. The eggs were emptied onto a Petri dish and counted. This count, in conjunction with the total weight of the egg sample yielded weight in eggs per g. A digital photograph was taken of each sample and analysed with Image Pro Express v. 4.5.1.3 (Media Cybernetics Inc., Bethesda, MD, USA). At Least 15 eggs per fish were then randomly selected and measured for a mean egg diameter.

### Biochemistry

For biochemical procedures, a portion of the egg sample (~1 g) belonging to an individual fish was subsampled. These subsamples were then combined and homogenized with the samples from all other fish from the same pond. This produced a total of nine 'pond homogenate' samples. The biochemical analyses that followed were conducted in triplicate on each of these homogenate samples. Biochemistry conducted included proximate analysis of protein, lipid and moisture. Protein was analysed by the micro-kjeldahl technique adapted from Ma & Zuazago (1942). Methods for lipid procedures were adapted from Folch *et al.* (1957) and AOAC (1990). For lipid extraction, 0.2–0.5 g of sample was homogenized in 6 mL of a 2 : 1 chloroform/methanol

solution. The sample was then filtered for solids and eluted by distilled water and stored for >6 h. After separation of the phases was complete, the upper 'waste' phase was removed, the lower phase was gently washed in triplicate with fresh 'upper phase' of 3 : 48 : 47 chloroform : methanol : water. The lower phase was then evaporated under nitrogen to dryness and weighed as total lipid. The lipid portion was then further analysed qualitatively by gas chromatography to determine the fatty acid composition.

The dried lipid extracts were then methylated and suspended in hexane. For analytical reference, a known concentration of C:19 was added to the sample. For gas chromatographic analysis, 0.1  $\mu$ L aliquots were used. The samples were analysed with a gas chromatograph (Model GC-17A; Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) equipped with a flame ionization detector and an Omegawax 530 capillary column (30 m  $\times$  0.53 mm; Supelco, Bellefonte, PA, USA). The initial temperature in the column was 140 °C, 2 min after the initiation of the analysis the temperature was increased at a rate of 3 °C min<sup>-1</sup> to a final temperature of 260 °C. Fatty acids were identified by comparing retention times against those of known standards and expressed as a percentage of total identified fatty acids.

### Data analysis

Data were assessed by an analysis of variation to determine significant ( $P < 0.05$ ) differences between the treatment means. In instances when individual female data could be followed, a nested analysis of variance was used where the individual data were nested by pond and analysed by treatment. The Student–Neuman–Keuls multiple comparison test was used to determine significant differences among treatment means (Steel & Torrie 1980). Spawning percentage, a binary response, was analysed with logistical analyses. All statistical analyses were conducted using SAS (V9.1; SAS Institute, Cary, NC, USA).

### Results

Brood stock size and condition are presented in Table 2 for the three treatment groups: standard feed (SD), forage fish supplementation (F) and topcoated diet (TC). The size of fish and their associated condition index were similar among treatments both at stocking and at harvest. Brood stock performance parameters are presented in Table 3. Brood stock performance was normalized in two ways: (i) weight of fish that spawned, and (ii) weight of fish that were harvested. The second value includes the weights of all the fish from the

**Table 2** Average initial and final length, weight and relative weight ( $W_r$ ) of brood fish fed three diets; a standard diet (SD) standard diet supplemented with forage fish (F) and a standard diet top coated with HUFAs (TC), in the spring prior to spawning ( $n = 72$  per each treatment)

Parameter	SD	F	TC	<i>P</i> -value <sup>1</sup>	PSE
Length (mm)					
Initial	577.6	580.5	581.0	0.048 (0.809, 0.019)	23.459
Final	612.2	613.7	615.2	0.024 (0.866, 0.009)	20.608
Weight (g)					
Initial	2211.0	2231.0	2287.8	0.100 (0.528, 0.061)	296.108
Final	2759.7	2737.8	2814.0	0.0017 (0.577, 0.001)	288.271
Relative weight ( $W_r$ )					
Initial	110.6	109.6	112.1	0.061 (0.227, 0.060)	6.419
Final	114.5	112.8	113.7	0.636 (0.539, 0.561)	6.335

PSE, pooled standard error.

<sup>1</sup> Analysis was performed using ANOVA models. Where data for individual fish were available, data were nested by ponds and three *P*-values are reported. The first value is that of the model. The two additional *P*-values presented in ( ) are those arising from the treatment and the pond (nested by treatment) respectively.

pond, regardless of whether they spawned or not. These values (with the exception of number of eggs per kg female that spawned) displayed a numerically increasing trend from SD through F to TC. Per cent spawn increased from 65% to 77%, fry production increased by ~600 fry kg<sup>-1</sup> brood fish, and per cent hatch increased from 40.6% to 47.4%. The size of the egg mass was found to be the only statistically distinct response to the topcoated diet ( $P = 0.003$ ) with an increase

from 138.3 to 155.9 g of eggs per kg female that spawned for the SD and TC treatments respectively.

Egg characteristics are presented in Table 4. Eggs size, both in diameter and in weight, increased with dietary lipid availability and those from TC were found to be significantly larger ( $P = 0.001$ ). Diameter and weights of the eggs were, on average, 3.5 mm and 18.9 mg, 3.6 mm and 19.1 mg and 3.8 mm and 21.4 mg, for SD, F and TC respectively. Proximate composition of these eggs however, did not follow the same trend. Eggs from across the treatments had similar moisture, protein and lipid levels (744–747, 159–164 and 53–57 g kg<sup>-1</sup> respectively) When these relative composition values are combined with the overall egg size, the mg component per egg results. These parameters show a numerical increase in value from SD, through F, to TC.

The total fatty acid composition of these eggs is presented in Table 5. The data indicate that there is not a significant difference in linoleic (18:2n6) or arachidonic (20:4n6) acids among treatments ( $P > 0.05$ ). EPA and DHA, however, were significantly greater in eggs from the TC treatment ( $P = 0.001$ ). Linolenic acid was also relatively higher in eggs from the F treatment ( $P = 0.021$ ). The total n-6/n-3 complement of the eggs was significantly altered; TC showing higher relative quantities of n-3 fatty acids, lower quantities of n-6 fatty acids and a consequentially shifted n-3/n-6 ratio ( $P < 0.05$ ). The differential incorporation of selected HUFAs across treatments is seen in Fig. 1.

**Table 3** Brood stock performance<sup>1</sup> and hybrid fry production of channel catfish females fed three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet top coated with HUFAs (TC), in the spring prior to spawning<sup>2</sup>

Parameter	SD	F	TC	<i>P</i> -value <sup>3</sup>	PSE
Per cent spawn <sup>4</sup>	65.2	74.5	77.3	0.533	–
Eggs per kg female spawned	7435	7141	7266	0.510 (0.910, 0.354)	455.1
g eggs per kg female spawned	138.3 <sup>b</sup>	135.3 <sup>b</sup>	155.9 <sup>a</sup>	0.003 (0.034, 0.010)	10.6
g eggs per total kg females harvested <sup>5</sup>	87.3	99.8	112.8	0.375	14.5
Fry per kg female spawned	2875	2933	3457	0.041 (0.197, 0.046)	439.9
Fry per kg females harvested	1787	2146	2370	0.454	439.7
Viable prehatch fry (%)	40.6	41.6	47.4	0.010 (0.270, 0.008)	5.8
Total fry	291 117	376 067	433 583		

PSE, pooled standard error.

<sup>1</sup> Brood stock performance was calculated both by the average weight of females that spawned (expressed eggs) and by the average of the total weight of fish harvested from that treatment regardless of egg expression.

<sup>2</sup> Mean values not sharing a common superscript within a row are significantly different ( $P < 0.05$ ) based on a Student–Neuman–Keuls multiple range test.

<sup>3</sup> Analysis was performed using ANOVA models. Where data for individual fish were available, data were nested by ponds and three *P*-values are reported. The first value is that of the model. The two additional *P*-values presented in ( ) are those arising from the treatment and the pond (nested by treatment) respectively.

<sup>4</sup> Per cent spawn was analysed as a binary response, using an exact logistic analysis.

<sup>5</sup> This value was calculated as the mean parameter normalized by the average body weight of the entire female brood stock population for that treatment, including those fish that did not express eggs.

**Table 4** Mean proximate composition of eggs from channel catfish females fed on three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet topcoated with HUFAs (TC), in the spring prior to spawning<sup>1</sup> ( $n = 50$  per each treatment)

Egg characteristic	SD	F	TC	<i>P</i> -value <sup>2</sup>	PSE
Mean egg weight (mg)	18.9 <sup>b</sup>	19.1 <sup>b</sup>	21.4 <sup>a</sup>	0.001 (0.001, 0.001)	0.01
Mean egg diameter (mm)	3.5 <sup>b</sup>	3.6 <sup>b</sup>	3.8 <sup>a</sup>	0.001 (0.001, 0.001)	0.14
Lipids (g kg <sup>-1</sup> )	53	57	54	0.109	0.49
Protein (g kg <sup>-1</sup> )	164	165	159	0.08	0.58
Moisture (g kg <sup>-1</sup> )	744	747	747	0.675	0.44
mg lipid per egg	1.0	1.1	1.2	0.305	0.17
mg protein per egg	3.1	3.1	3.4	0.351	0.10

PSE, pooled standard error.

<sup>1</sup> Mean values not sharing a common superscript within a row are significantly different ( $P \leq 0.05$ ) based on a Student–Neuman–Keuls multiple range test.

<sup>2</sup> Analysis was performed using ANOVA models. Where data for individual fish were available, data were nested by ponds and three *P*-values are reported. The first value is that of the model. The two additional *P*-values presented in ( ) are those arising from the treatment and the pond (nested by treatment) respectively.

The fatty acid profiles of the polar and neutral fractions of the egg lipids are presented in Tables 6 & 7. The trends of HUFA incorporation in egg lipids seen in the previous tables and figures are mirrored in the polar fraction. The TC treatment had greater DHA and total n-3 incorporation, lower total n-6 abundance and a shifted n-3/n-6 ratio in the polar component (Table 6). The HUFA content of the neutral fraction was minimal and displayed few strong trends between treatments, thus it was not highlighted further.

The relative abundance of ARA, EPA and DHA in both the feed and the eggs from the SD and TC treatments is presented in Fig. 2. Increased proportions of EPA and DHA in the diet were correlated with increased relative quantities of these molecules in the egg. The increased availability of ARA in the TC diet did not correlate to an increased incorporation of ARA into the eggs from this treatment.

## Discussion

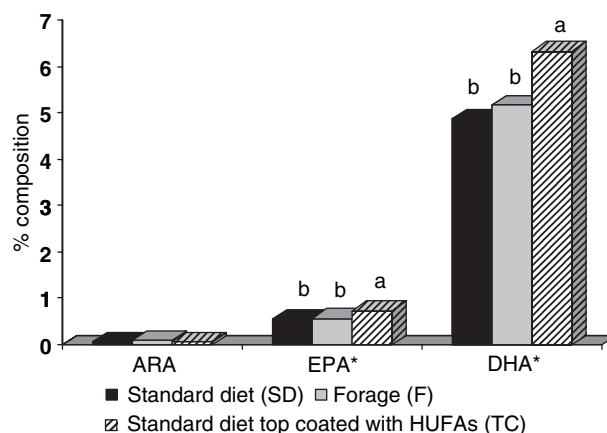
The large scale incorporation of hybrid catfish into the commercial catfish industry requires a dependable and economical source of fingerlings. One way to increase the performance of the brood stock and survival of the embryos is to provide the females with a lipid enhanced diet to increase egg production and quality. Results from this study suggest that dietary lipid supplements offered to female channel catfish

**Table 5** Mean percentage ( $\pm$  standard deviation) fatty acid composition of channel catfish eggs fed three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet topcoated with HUFAs (TC) in the spring prior to spawning<sup>1</sup> ( $n = 9$  per each treatment)

Fatty acid	SD	F	TC	<i>P</i> -value
C14:0	0.66 $\pm$ 0.017	0.67 $\pm$ 0.009	0.70 $\pm$ 0.019	0.156
C16:0	16.10 $\pm$ 0.131	15.80 $\pm$ 0.089	16.14 $\pm$ 0.061	0.041
C16:1	2.31 $\pm$ 0.035 <sup>b</sup>	2.45 $\pm$ 0.026 <sup>a</sup>	2.28 $\pm$ 0.032 <sup>b</sup>	0.002
C18:0	13.35 $\pm$ 0.729 <sup>a</sup>	14.01 $\pm$ 0.454 <sup>a</sup>	11.66 $\pm$ 0.294 <sup>b</sup>	0.012
C18:1n9	28.81 $\pm$ 0.886	28.01 $\pm$ 0.564	29.88 $\pm$ 0.306	0.132
C18:2n-6	8.93 $\pm$ 0.173 <sup>b</sup>	9.38 $\pm$ 0.095 <sup>a</sup>	8.83 $\pm$ 0.131 <sup>b</sup>	0.021
C19:0 <sup>2</sup>	7.23 $\pm$ 0.606	6.28 $\pm$ 0.273	7.94 $\pm$ 0.565	0.086
C18:3n-3	0.49 $\pm$ 0.029	0.49 $\pm$ 0.017	0.50 $\pm$ 0.009	0.962
C20:1n-9	1.24 $\pm$ 0.018	1.27 $\pm$ 0.011	1.22 $\pm$ 0.018	0.097
C20:3n6	3.40 $\pm$ 0.096 <sup>b</sup>	3.64 $\pm$ 0.037 <sup>a</sup>	3.10 $\pm$ 0.062 <sup>c</sup>	0.001
C20:3n-3	6.94 $\pm$ 0.164 <sup>a</sup>	7.09 $\pm$ 0.118 <sup>a</sup>	6.44 $\pm$ 0.102 <sup>b</sup>	0.005
C20:4n6	0.06 $\pm$ 0.010	0.09 $\pm$ 0.011	0.06 $\pm$ 0.006	0.055
C20:5n-3	0.55 $\pm$ 0.017 <sup>b</sup>	0.56 $\pm$ 0.004 <sup>b</sup>	0.73 $\pm$ 0.015 <sup>a</sup>	0.001
C22:5n6	2.22 $\pm$ 0.062 <sup>a</sup>	2.20 $\pm$ 0.049 <sup>a</sup>	1.61 $\pm$ 0.041 <sup>b</sup>	0.001
C22:5n3	0.62 $\pm$ 0.015	0.61 $\pm$ 0.010	0.60 $\pm$ 0.012	0.685
C22:6n3	4.88 $\pm$ 0.120 <sup>b</sup>	5.17 $\pm$ 0.063 <sup>b</sup>	6.34 $\pm$ 0.134 <sup>a</sup>	0.001
$\Sigma$ n-6	14.61 $\pm$ 0.323 <sup>b</sup>	15.31 $\pm$ 0.130 <sup>a</sup>	13.60 $\pm$ 0.228 <sup>c</sup>	0.001
$\Sigma$ n-3	13.48 $\pm$ 0.323 <sup>b</sup>	13.94 $\pm$ 0.181 <sup>ab</sup>	14.61 $\pm$ 0.256 <sup>a</sup>	0.018
n-3/n-6	0.92 $\pm$ 0.006 <sup>b</sup>	0.91 $\pm$ 0.010 <sup>b</sup>	1.07 $\pm$ 0.005 <sup>a</sup>	0.001

<sup>1</sup> Mean values not sharing a common superscript within a row are significantly different ( $P \leq 0.05$ ) based on a Student–Neuman–Keuls multiple range test.

<sup>2</sup> C:19 was added as an indicator molecule for analysis by gas chromatography.



**Figure 1** Relative per cent composition of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the eggs from female channel catfish brood stock fed three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet topcoated with HUFAs (TC) in the spring prior to spawning. \*Variables that were found to be statistically significant ( $P < 0.05$ ) between treatments.

**Table 6** Mean per cent fatty acid content in the polar fraction of channel catfish eggs fed three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet topcoated with HUFAs (TC) in the spring prior to spawning<sup>1</sup> ( $n = 9$  per each treatment)

Fatty acid	SD	F	TC	P-value
C14:0	0.13 ± 0.137	0.10 ± 0.068	0.11 ± 0.068	0.845
C16:0	11.41 ± 0.300	11.44 ± 0.491	11.65 ± 1.00	0.724
C16:1	0.55 ± 0.023 <sup>b</sup>	0.61 ± 0.230 <sup>a</sup>	0.56 ± 0.035 <sup>b</sup>	0.013
C18:0	18.93 ± 0.669	19.40 ± 1.17	18.85 ± 1.241	0.515
C18:1n9	21.82 ± 0.723	21.71 ± 1.630	22.18 ± 1.923	0.801
C18:2n-6	6.35 ± 0.409 <sup>b</sup>	6.91 ± 0.406 <sup>a</sup>	6.21 ± 0.430 <sup>b</sup>	0.004
C19:0 <sup>2</sup>	6.55 ± 1.213	5.62 ± 0.691	4.38 ± 3.022	0.090
C18:3n-3	0.29 ± 0.101	0.29 ± 0.071	0.27 ± 0.029	0.754
C20:1n-9	1.20 ± 0.045	1.29 ± 0.100	1.23 ± 0.069	0.075
C20:3n6	4.18 ± 0.208	4.12 ± 1.347	4.04 ± 0.259	0.932
C20:3n-3	12.13 ± 0.298	10.88 ± 2.215	12.28 ± 0.646	0.082
C20:4n6	0.18 ± 0.098	0.17 ± 0.033	0.15 ± 0.044	0.570
C20:5n-3	0.79 ± 0.034	1.08 ± 0.630	1.20 ± 0.101	0.100
C22:5n6	3.61 ± 0.180 <sup>a</sup>	3.63 ± 0.484 <sup>a</sup>	2.68 ± 0.264 <sup>b</sup>	0.001
C22:5n3	1.87 ± 1.914	1.74 ± 0.709	1.15 ± 0.120	0.384
C22:6n3	8.10 ± 0.383 <sup>c</sup>	9.13 ± 1.191 <sup>b</sup>	11.08 ± 0.793 <sup>a</sup>	0.001
Σn-6	14.33 ± 0.705 <sup>a</sup>	14.83 ± 1.076 <sup>a</sup>	13.07 ± 0.920 <sup>b</sup>	0.002
Σn-3	23.18 ± 1.692 <sup>b</sup>	23.12 ± 1.592 <sup>b</sup>	25.98 ± 1.406 <sup>a</sup>	0.001
n-3/n-6	1.62 ± 0.179 <sup>b</sup>	1.56 ± 0.094 <sup>b</sup>	1.99 ± 0.073 <sup>a</sup>	0.001

<sup>1</sup> Mean values not sharing a common superscript within a row are significantly different ( $P < 0.05$ ) based on Student–Neuman–Keuls multiple range test.

<sup>2</sup> C:19 was added as an indicator molecule for analysis by gas chromatography.

brood stock in the spring before spawning are incorporated into the egg and that these supplements tended to correlate with higher brood stock performance and egg quality (Tables 3 & 4). While ovodeposition in channel catfish may begin in fall (Brauhn & McCraren 1975; Newman 1990), the data presented here suggest that the lipid profile of the egg, at least, can still be affected by the diet of the fish within 3 months prior to spawning. This is exemplified by a reflection of the similarities and the differences in the lipid profiles of the eggs as compared to the diets.

The increased availability of HUFAs in the diet correlated with an increased incorporation of these molecules into the egg (Fig. 2). The physiological ability to elongate PUFAs (found readily in the control diet) into HUFAs is apparent, with a significant accumulation of HUFAs and n-3 fatty acids in all treatments, regardless of the lipid source (Table 5 and Fig. 2). The relative proportion of these HUFAs, however, was affected by the type of dietary supplementation, suggesting a limitation in the diet or in the physiological ability to modify fatty acid precursors into these molecules. Given the complex pathway of lipid digestion, allocation and

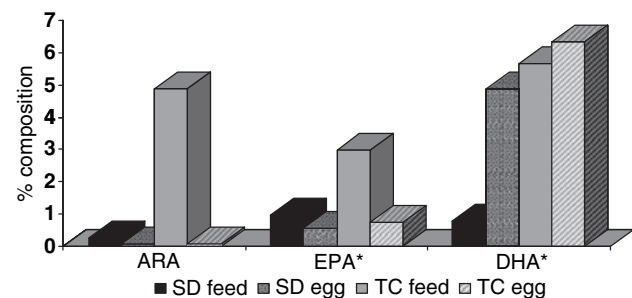
**Table 7** Mean per cent fatty acid content in the neutral fraction of eggs from female channel catfish fed three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet top coated with HUFAs (TC) in the spring prior to spawning<sup>1</sup> ( $n = 9$  per each treatment)

Fatty acid	SD	F	TC	P-value
C14:0	1.33 ± 0.705	2.36 ± 1.824	2.54 ± 1.641	0.188
C16:0	22.86 ± 1.097	22.94 ± 1.081	23.92 ± 2.509	0.352
C16:1	2.92 ± 0.329 <sup>b</sup>	3.24 ± 0.323 <sup>a</sup>	3.47 ± 0.223 <sup>a</sup>	0.003
C18:0	3.97 ± 0.200	3.99 ± 0.326	3.81 ± 0.200	0.26
C18:1n9	33.86 ± 1.577	34.17 ± 1.609	34.92 ± 3.989	0.684
C18:2n-6	9.41 ± 0.636 <sup>b</sup>	10.09 ± 0.973 <sup>b</sup>	11.44 ± 1.042 <sup>a</sup>	0.001
C19:0 <sup>2</sup>	16.09 ± 2.907 <sup>a</sup>	13.99 ± 2.064 <sup>ab</sup>	10.50 ± 7.042 <sup>b</sup>	0.048
C18:3n-3	0.46 ± 0.078 <sup>b</sup>	0.51 ± 0.068 <sup>ab</sup>	0.60 ± 0.119 <sup>a</sup>	0.014
C20:1n-9	1.12 ± 0.034	1.14 ± 0.110	1.17 ± 0.077	0.411
C20:3n6	1.77 ± 0.068 <sup>b</sup>	1.92 ± 0.172 <sup>a</sup>	1.97 ± 0.169 <sup>a</sup>	0.023
C20:3n-3	0.92 ± 0.078	1.04 ± 0.233	0.936 ± 0.119	0.232
C20:4n6	ND	ND	ND	–
C20:5n-3	ND	ND	ND	–
C22:5n6	ND	ND	ND	–
C22:5n3	ND	ND	ND	–
C22:6n3	1.11 ± 0.523	1.53 ± 1.074	1.24 ± 0.377	0.466
Σn-6	11.19 ± 0.694 <sup>b</sup>	12.01 ± 1.129 <sup>b</sup>	13.41 ± 1.209 <sup>a</sup>	0.001
Σn-3	2.49 ± 0.594	3.08 ± 1.081	2.78 ± 0.329	0.259
n-3/n-6	0.22 ± 0.057	0.26 ± 0.099	0.21 ± 0.033	0.301

ND, components that were below detectable limits.

<sup>1</sup> Mean values not sharing a common superscript within a row are significantly different ( $P < 0.05$ ) based on Student–Neuman–Keuls multiple range test.

<sup>2</sup> C:19 was added as an indicator molecule for analysis by gas chromatography.



**Figure 2** Average relative percentage of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the feed and the eggs of channel catfish fed two diets: a standard diet (SD) and a standard diet topcoated with HUFAs (TC) in the spring prior to spawning. \*HUFAs molecules for which an increased availability in the diet correlated with an increased incorporation in the egg.

vitellogenic inclusion, it is unlikely that this increased incorporation is a passive or ‘diffusive’ response to increased dietary abundance. High quality dietary lipid limitations are well documented and researched in marine species

(Watanabe 1993; Sargent 1995; Wiegand 1996; Brooks *et al.* 1997; Tveiten *et al.* 2004) but the converse is true for fresh-water species, whose lipid metabolism is more complex. Gatlin & Stickney (1982) demonstrated that HUFA-supplemented diets had little effect on the growth rates of juvenile channel catfish, although their tissue composition strongly reflected the oil source in the diet. The effect of these dietary lipids upon the female brooders seen here, however, suggests that a limitation in HUFA availability for incorporation into the eggs may be present in typical commercial pond settings.

The lipid component of a fish egg is functionally, chemically and conceptually separated into the polar and neutral fractions. The neutral fraction, typically rich in triacylglycerols and wax esters, is the predominant class of lipid molecules found in the energetic reserves of the eggs, such as the oil droplet (Wiegand 1996; Tveiten *et al.* 2004). The polar fraction, frequently replete in 'structural lipids' such as lipoproteins and phospholipids, can account for 50–90% of the total lipid complement and contains most of the lipid molecules used in membranes and cell signalling. The distinctly higher proportion of HUFAs in the polar fraction from the egg samples analysed here highlights the importance of these molecules as essential structural and developmental components. It is not surprising then, that the trends of incorporation for these HUFAs seen in the total lipid samples are reflected, almost identically, in the polar fraction. The relative abundance of n-3 fatty acids in the polar fraction surpasses that in the neutral fraction by nearly a factor of 10. The n-6 fatty acids, whose developmental importance is less clear (Sargent *et al.* 1999; Tveiten *et al.* 2004), showed the opposite trend with an increased incorporation of these molecules into the neutral (energetic) fraction as their dietary availability increased (Table 7).

Brood stock performance, in terms of egg and fry production, was less affected by dietary enhancement than egg quality parameters. While there were apparent numerical trends across nearly all parameters, these tendencies, while perhaps functionally important, lacked statistical significance (Table 3). The same is true for the proximate composition of the eggs across treatments (Table 4). Where these data arise from individual fish measurements, the statistical analyses of these variables frequently indicate that a high degree of variation on the pond, not treatment, level was at least partially responsible for this lack of distinction.

Another contributor to the lack of treatment differences lies in the quality and nutritional background of the brood stock. In this study, the fish were of high quality ( $W_r = 110$ – $112$ ) and relatively uniform across the total population

(Table 2). Previous studies by Quintero (2007) showed channel catfish female brood stock to have a much more distinct response in performance to lipid-supplemented diets using brooders that were, on average, of much poorer quality ( $W_r = 65$ – $80$ ). At the termination of both treatments, however, the final condition was both high and relatively similar ( $W_r \approx 112$ – $125$ ) between the two experiments. This improved condition and response to a lipid-enriched diet suggest that this type of feed may have a more profound effect as a brood stock rehabilitation treatment. As a poor quality female brood fish nears the spawning season, perhaps a high quality diet, rich in HUFAs, can be rapidly utilized and incorporated into the oocytes to compensate for any previous nutritional deficiencies.

Channel catfish are typically reared on a diet formulated, for various reasons, to minimize fish oil incorporation. Fish oils are effectively replaced with vegetable oils to satisfy the demands, energetic and structural, of this species (Gatlin & Stickney 1982). In the commercial management of catfish brood stock, fish are provided an optimum environment in which to mature, accumulate energy and nutrients and develop oocytes. The nutrition of these fish, however, typically remains unchanged or only slightly augmented (Kelly 2004). A diet rich in PUFAs and low in HUFAs such as a commercial feed (PUFA : HUFA ratio in SD  $\approx 14 : 1$ ) is sufficient for growing catfish (Gatlin & Stickney 1982), but it may be insufficient for the optimal maturation of channel catfish ovaries, as suggested by the trends seen here. To supply the eggs with an ideal complement of high-quality lipid molecules, the female will acquire, biochemically alter and allocate a considerable quantity of the available resources into the oocyte. This is represented by the fish in the SD treatment whose HUFA complement of the eggs was dramatically increased compared to the diet (Fig. 2). When female channel catfish brood stock are fed a commercial ration, there appears, however, to be a functional limit to their ability to elongate dietary PUFA precursors into HUFAs for incorporation into the egg.

Beyond this synthetic limit, the only way to increase HUFA incorporation in the egg is through the dietary supplementation of the parent. One way which HUFAs can be delivered is through stocking forage fish (Santiago 1979; Torrains & Lowell 2001; Kelly 2004). Forage fish such as sunfish and fathead minnows bio-accumulate PUFAs from the environment and act as a natural prey item rich in ARA, EPA and DHA (Brett & Muller-Navarra. 1997; Oster 2002; USDA 2006). While this type of lipid supplementation has previously shown a marked improvement in egg and brooder quality (Torrains & Lowell 2001), the affects of forage fish in

this study were overshadowed by the diet with a direct supplementation of menhaden fish oil, ARA and DHA.

Dietary lipids and their effect on egg quality are the understudied facets of freshwater finfish nutrition. Due to the complex internal and environmental biochemical pathways associated with these molecules in a freshwater environment, their effect is more difficult to approximate than in marine species. Without this research, the added cost of a higher quality feed may not be justified in all situations. The typical production practices of a channel catfish hatchery involve pond spawning, reasonable hatch rates and limited investment (Steeby & Avery 2005). For this setting, the added cost of a high HUFA diet may not be warranted in all cases. For hybrid catfish production, involving hand stripping, incubation, low hatch rates and high investment, however, a high quality brood stock diet may be necessary investment if the return is ~600 or more fish per kg of female (Table 2). Additionally, this high cost feed appears to affect significantly the females even when applied in the spring, albeit late into the oocyte development period. This response also seems to be accentuated in a population of females of suboptimal quality. The added cost of this feed over a short treatment window can be weighed against the benefits of increasing the general reproductive output of a group of female brooders to determine if its application is justified.

The results of this study indicate that increasing the dietary availability of HUFAs through a topcoated diet significantly increased: the HUFA complement of the egg (Tables 5 & 6, Figs 1 & 2), the egg size, both weight and diameter (Table 4) and the total egg mass size per kg female brooder that spawned (Table 3). The addition of forage fish to the brood stock ponds at ~28 kg ha<sup>-1</sup> had no significant effect compared to a commercial feed alone ( $P < 0.05$ ) (Tables 3–7) except in the DHA relative abundance in the polar fraction of the egg lipids (Table 6). The effects of both the F and TC diets upon many parameters such as per cent spawn and egg survival were numerically apparent but statistically unclear due to a high degree of variability on the pond level within the treatments (Tables 3 & 4).

There are many aspects of the HUFA complement that have yet to be studied in freshwater diets. The relative concentration between HUFA molecules (such as ARA : EPA) has been suggested to be an additional highly important factor in egg quality and survival through hatch (Sargent *et al.* 1999; Tveiten *et al.* 2004). The data from this study, however, had no significant correlation between HUFA content or ratios and egg survival. The differential incorporation of PUFA types into the polar and neutral fractions

along with the egg in general does offer some insight. In the TC diet, ARA was present in high quantities (4.88%) (Table 1 and Fig. 2) but unlike other HUFA molecules it appeared in the egg only in trace amounts (0.064%) (Table 5 and Fig. 2). Similarly, the neutral fraction of the lipids from eggs in the TC treatment had significantly increased levels of n-6 fatty acids (Table 6). These two facts support the theory that HUFAs and n-3/n-6 ratio are important factors in egg quality and are closely regulated in the oocyte.

This study indicates that performance of catfish brood stock is related to the quality and nutritional background of the brood stock, and may be enhanced with appropriate diets during the spring season. Thus, enriching the commercial diet directly with a lipid topcoat rich in HUFAs had a greater effect on increasing the egg quality and brooder performance than stocking natural prey items at 28 kg ha<sup>-1</sup>. In the production of hybrid catfish, the female brood fish management programme should seek to provide an artificial diet with an increased HUFA complement to increase overall fecundity and fry production from a population. By increasing the reproductive quality of the female brood stock, the hatchery can increase the total economic efficiency of hybrid fry production.

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