AN EVALUATION OF NATURAL AND ARTIFICIAL DIETARY LIPID SOURCES

ON EGG QUALITY AND FRY PRODUCTION IN CHANNEL CATFISH (♀) x

BLUE CATFISH (♂) HYBRIDIZATION

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Evan R. Durland

Certificate of Approval:

_____	_____
Rex A. Dunham Alumni Professor Fisheries and Allied Aquacultures	D. Allen Davis, Chair Associate Professor Fisheries and Allied Aquaucultures
_____	_____
Ronald P. Phelps Associate Professor Fisheries and Allied Aquacultures	Joe F. Pittman Interim Dean Graduate School

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Evan R. Durland

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Evan R Durland

_____
Signature of Author

_____
Date of Graduation

VITA

Evan R. Durland, son of Eric and Brooke and brother of Garrett, was born February 24th 1982 in Denver, Colorado. He studied marine biology at James Cook University in Queensland, Australia and Colorado State University. In 2004, he received a Bachelors of Science, with honors, from the latter. After a year of working as a hatchery technician in Colorado, travelling internationally, and paddling across Canada he enrolled in Auburn University to pursue a Masters of Science degree in Aquaculture in the Department of Fisheries and Allied Aquacultures.

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Hybrid catfish (channel catfish *Ictalurus punctatus*♀ x blue catfish *I. furcatus*♂) display many characteristics that are favorable to aquaculture production such as increased growth, resistance to disease, higher dress out and ease of seining. The implementation of this hybrid variety into full scale aquaculture production is limited mainly by the difficulties involved with spawning, incubation and larval rearing. One of the confounding stages that has high mortality rates is the complex developmental steps that take place after fertilization. In this phase, the developing embryo must obtain its nutrients and structural components for development from the egg reserves. Therefore, to maximize the survival of these hybrid embryos through hatch, a high quality egg should be supplied by the female. This study was

conducted in an attempt to isolate nutritional lipids that lead to high quality egg production in female channel catfish. A 10 week feed trial was conducted in ponds in Auburn, Alabama. In March, 219 female channel catfish brood stock were stocked into nine ponds 0.04 ha in size each for an approximate stocking rate of 1,332 kg/ha. Three dietary treatments were randomly allocated to the fish. Diet-1 was a standard 32% crude protein, 6% lipid floating catfish feed. Diet-2 was the same feed supplemented with forage fish at approximately 28 kg/ha. The third diet was the aforementioned catfish feed top-coated with 2% lipid (1% Menhaden fish oil, 0.5% high DHA oil and 0.5% high ARA oil). The fish were fed the prepared feeds three times a week at 1.5% body weight per feeding. Dissolved oxygen and temperatures were measured twice daily at dawn and dusk, and low DO events were mitigated by nighttime aeration. Ammonia, nitrite and pH parameters were measured twice a week. In May, the females were harvested, administered injections of luteinizing hormone releasing hormone analog to induce ovulation and strip spawned. The eggs were fertilized with blue catfish sperm and incubated in paddle wheel troughs. Percent viable fry was estimated by egg mass assessments 24 hours prior to hatch and resultant fry counts. The spawning data indicates brood fish fed the high lipid diet spawned larger egg masses (+17.6 g eggs/kg brood fish, $p=0.003$) and had larger eggs both in weight and diameter, when compared to either the control or forage fish treatment (+2.5mg and +0.3mm, $p=0.001$). These eggs, in turn, had increased complements of high quality lipids such as DHA, EPA and total n-3 fatty acids ($p=0.001$).

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This study was funded by a grant from the Southern Regional Aquaculture Center and was greatly assisted by Dr. Dunham and his research crew who, in the spring, I am convinced never sleep. To Dr. Phelps I am grateful for helping me understand fish reproduction and entertaining my endless questions. My appreciation goes out to these and all the other people that helped me with the physical and intellectual challenges in this study.

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I. INTRODUCTION

Channel catfish aquaculture is an industry in the United States dating back to the 1930's. Production has increased to a total of ~263,000 metric tons in 2006 worth an estimated $481 million dollars (USDA, 2007). In recent years, the foreign production and importation of catfish fillets has increased to substantial levels. In the three year period between 1997 and 2000, imported catfish fillets increased eight-fold from ~430 mt to ~3,700 mt. The introduction of these imported products and loss of market share have negatively impacted the farm-gate price for domestic producers (Quagraine and Engle, 2002).

One way for farmers to increase production and bolster their profit margin is by choosing an organism with characteristics that respond well to the increasingly intense production systems of the catfish industry. This selection of fish includes different species, genetic strains, and hybrids. While a few producers still stock blue catfish, the vast majority of research and commercial production in the United States is with channel catfish (*Ictalurus punctatus*). Within this species are myriad strains that have been collected and developed from different parts of the continent, each displaying unique attributes. Over the years these strains have been selected and crossed successfully to produce high performance varieties of channel catfish with advantageous traits such as faster growth, higher fecundity and increased disease resistance (Smitherman et al. 1978, Dunham 1979; Youngblood 1980; Dunham et.al, 1983, Dunham and Brummett, 1999).

In an effort to investigate and enhance favorable catfish characteristics, channel catfish females have been crossed with blue catfish (*Ictalurus furcatus*) males to produce hybrids. 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 and Argue, 1998, Dunham and Argue, 2000, Argue et. al., 2003). These advantageous traits, however, are limited almost exclusively to the F1 generation. Hybrids of the F2 generation and those resulting from an F1 x parent backcross show a substantial loss of the hybrid vigor witnessed in the F1 populations (Dunham and Argue, 2000).

Induced Spawning of Catfish

For all the advantageous traits of this F1 hybrid generation, they are rarely cultured due to limited fingerling availability resulting from difficulties involved with spawning, fertilization and survival to hatch (Dunham and Smitherman, 1984, Dunham et. al., 1999). The occurrence of "natural hybridization" between these species is sporadic at best and thus, to reliably produce the hybrid catfish, the females must be brought into a hatchery, hormone induced, strip spawned and manually fertilized with blue catfish sperm. Channel catfish are traditionally spawned in ponds, the eggs collected from spawning cans or containers and incubated in hatcheries. The methodology of induced spawning for this species has been developed over the years for situations where more control over the reproduction of these animals is desired such as for genetic experiments (Sneed and Clemens, 1959, Busch, 1985, Dunham, 1993). This method of spawning can potentially increase the fry production of a

group of fish but has significant costs arising from the required facilities, labor and the sacrificing of males for sperm. The increased fry production should, however, compensate for the investment of dollars and man hours. In hybrid catfish production, this is not always the case. Pure channel catfish spawnings have traditionally shown hatch percentages double those of the hybrid crosses (Dunham and Argue, 2000). This limited return on the investment drives the price of these fry up to a point that becomes uneconomical or prohibitive for farmers to purchase and stock them.

In an attempt to improve the fry production of this species, Dunham et al. (1999) conducted experiments with different strains of channel catfish and fertilization techniques. This research indicated 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 likely associated with embryonic development within the egg after fertilization.

Egg Quality and Embryonic Development

The embryonic development of channel catfish was first comprehensively studied by Saksena et al. (1961). They reported that, with respect to other genera of catfishes, channel catfish have a rapidly developing embryo, with many structures and some musculature visible within 30 hours of fertilization at ~25ºC. The time to hatch for channel catfish eggs is variable with temperature, but usually averages between 5-7 days. Research comparing

the developmental rates of channel, blue and hybrid catfishes was examined by Campbell (1999). His research indicated that, of the three observed species, channel catfish embryos develop the most rapidly, blue catfish the slowest and that the hybrid organisms progressed at a rate somewhere between the two.

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 total composition of an egg is extremely complex and not entirely understood. Within the egg is an array of factors including DNA, RNA, enzymes, hormones, ions, proteins, lipids and combinations of all of the above provided by he female parent (Wiegand, 1996, Brooks et al., 1997). The factors influencing the parent and thus the egg composition are numerous; genetics, stress, water quality, photoperiod, diet, age of the fish, and life history are only a few. In a well developed brood stock management program, all the controllable elements are optimized: stocking density is low, water quality is high, spawning period is predicted and utilized, fish are of good quality and the diet is comprehensive and complete. Many of these factors are quantitative, discrete and manageable, but the diet of brood stock is one factor that can be complex, confounding and simultaneously have a profound affect on egg quality (Brooks et al, 1997, Izquierdo et al., 2001).

Egg quality can be considered from many different perspectives including weight, diameter, color, biochemical composition or ultimately; survival through to first feeding of the embryo. Biochemically speaking, an egg of high quality is one with a full complement of nutritional and genetic material and a complete hormone/enzyme "startup package"(Brooks et al.,1997). From a nutritional prospective then, egg quality can be

considered as the protein, lipid, carbohydrate and vitamin profile (Wiegand,1996). The lipid component of this nutritional profile is both important to the developing embryo and frequently perplexing. In the developing embryo, lipid molecules serve a dual function; as general metabolic substrates as well as specific and essential components of new tissues (Sargent, 1995, Wiegand, 1996, Brooks et al., 1997). The types of lipids used for each of these functions can be quite different. While most fatty acid molecules can be catabolically consumed for energy, only a few are suitable as specific components in cell construction and differentiation. Cell membranes, especially those of neurons, are rich in n-3 poly unsaturated fatty acids (PUFAs) and the same class of molecules are likewise found to be important in eicosanoid production and cell membrane fluidity (Sargent, 1995, Wiegand, 1996).

PUFAs are a family of long chain lipid molecules with multiple double bonds along the carbon backbone. These structures can be categorized by their backbone length, the amount of un-saturation (double bonds) along that backbone and the location of the terminal (methyl) double bond. Linoleic acid, whose chemical structure is 18:2n6 is18 carbons long with two double bonds, the terminal one being 6 carbons removed from the methyl end. If the molecule contains 4 or more double bonds, the molecule may alternately be categorized as a highly unsaturated fatty acid (HUFA). These lipids are complex, specialized and frequently associated with very specific tissues and functions. In trout, it was observed by Leray et al. (1985) that eggs lacking a complement of these high quality lipid molecules had significantly higher mortalities during development and displayed disorders in cellular association. The significant difference between lipid molecule classes is reflected by the tendency for developing embryos to conserve long chain, unsaturated fatty acid molecules

for structural uses rather than to burn them for metabolic energy (Wiegand, 1996, Tveiten et al., 2004). To maximize the survival of a developing embryo, especially one that has been hybridized and struggles through early life stages, the eggs to be used should contain a sufficient quantity and quality of lipids and essential nutrients to facilitate the rapid development of early life stages (Brooks et al., 1997, Furuita et al. 2000, Chong et al. 2004).

Ovodeposition and Brood stock Nutrition

Oocyte development depends not only on the ingredients that can be synthesized by the parent, but other essential ingredients such as amino and fatty acids that cannot be synthesized *de novo*. These molecules must be obtained and accumulated rather than constructed. Therefore, the diet of the female brood fish determines, in part, the nutritive profile of the egg. It has been demonstrated by Fernandez-Palacios et al., 1995, Sargent, 1995, Brooks et al., 1997, Mokoginta, 1998, Sargent et al., 1999, Izquierdo et al., 2001 that a brood fish fed a high quality diet will produce high quality eggs that show improved rates of hatch and survival. Additionally, in aquaculture conditions, of the array of nutritional requirements for brood stock, it has been suggested that lipid composition is paramount in determining egg quality. Artificial diets are typically lacking a full complement of high quality lipid sources that are commonly found in the fish's natural foods (Sargent, 1995, Brooks et al., 1997).

HUFAs are a class of lipid molecules that are relatively special in nature, requiring a significant investment of energy in their creation. Their origin is almost exclusively from algae, from which they work their way up through the food chain to crustaceans and fish of

many trophic levels (Brett and Muller-Navarra, 1997). These molecules may be altered in configuration by cell machinery, but they cannot be synthesized entirely (except in rare cases) by higher animals. In aquatic animals, these lipids are acquired from the environment and incorporated structurally into tissues.

In freshwater fish, some PUFAs can be modified to create new, more complex varieties such as HUFAs. This anabolic process of chain elongation, whereby carbon atoms are attached to an existing skeleton is unique to some genera of freshwater fish, including channel catfish. The mechanics and capacities of this synthesis are not well understood but it seems that while molecules may be elongated and de-saturated (at a limited rate), the location of the "omega" or terminal double bond cannot be changed. This means that "families" of fatty acids can be created from one parent molecule. Omega-3 (n-3) fatty acids cannot be changed to omega-6 (n-6) fatty acids but linolenic acid (18:3n3) can be elongated to eicosapentaenoic acid (20:5n3) by the addition of 2 carbon atoms and two double bonds. The backbone may be elongated and kinked, but the tail remains the same.

This ability to synthesize complex lipid molecules from relatively less complex varieties is one reason why plant oils, which are high in PUFAs, can be used in growout diets of many freshwater fish. Conversely, marine fish have a more limited ability for chain elongation and thus fish oils, which are rich in HUFAs, are often used (Gatlin and Stickney, 1982, Ibeas et al, 1994., Francis et al., 2006). For the majority of the life cycle of the catfish, it seems that the synthetic modification of PUFAs to HUFAs is sufficient to support growth and metabolism. During times of high demand for these molecules such as early life stages or gonadal deposition, however, this source may be insufficient (Navas et al, 1997, Tveiten

et al., 2004). While the individual egg requirement of these high quality nutrients is small, the ovary size in a pre-spawn channel catfish can account for up to 15% of her total body weight and thus the total requirement can be substantial. Lipid inadequacies in a brood stock diet may have an affect on ovodeposition and vitellogenesis thus limiting the egg in these essential fatty acid reserves (Brauhn and McCraren, 1975, Sargent J.R., 1995, Brooks et al., 1997, Furuita et al., 2000, Chong et al., 2004).

Dietary sources of lipids

The need for high quality lipid sources in the brood stock diets of fish has not gone overlooked, but the majority of the interest and research for this requirement has been for marine rather than freshwater species (Fernandez-Palacios et al., 1995, Navas et al., 1997, Watanabe, 1997, Furuita et al., 2000). Santiago (1979) was one of the first to attempt to measure the affect of forage fish on the performance of catfish brood stock. While his research was inconclusive as to the necessity of forage fish supplementation, common practice (Kelly, 2004) and subsequent research such as that done by Torrans and Lowell (2001) has shown that a forage fish supplemented diet increases the egg and fry production of female channel catfish.

While forage fish and fish oils supply a wide array of HUFAs and PUFAs, it can be argued that there are a few specific HUFAs that are especially important in development. Of particular interest are arachidonic acid (20:4n6) (ARA), eicosapenaenoic acid(20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA) (Watanabe, 1993, Fernandez-Palacios et al., 1995, Navas et al., 1997, Sargent et al., 1999, Izquierdo et al., 2001). These molecules

have been demonstrated to be paramount in the array of HUFAs required for development from fertilization through juvenile stages.

Consequently, the objective of this study was to determine if 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 percent hatch of their hybrid offspring.

II. METHODS AND MATERIALS

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, approximately 219 four 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 1,332 kg/ha per pond. These fish were obtained from Jubilee Farms, MS. Three dietary treatments were developed and randomly assigned to each of the nine ponds, allowing for three replicates per treatment. Treatment 1 was a standard 32% crude protein, ~6% lipid floating catfish feed. Treatment 2 was the same feed supplemented with forage fish (bluegill and fathead minnows) at approximately 28 kg/ha, each. Treatment 3 was the standard catfish feed top-coated with 2% lipid (1% Menhaden fish oil, 0.5% high DHA oil and 0.5% high ARA oil). The DHA and ARA oils contained approximately 40% of the designated highly unsaturated fatty acid. Biochemical analyses of the feeds that were used are presented in Table 1. The standard and top-coated diets contain 9.4%, 10.4%, moisture; 32.9%, 32.2% protein, 5.95%, 7.58% fat, 5.06%, 4.64% fiber and 7.05%, 6.74% 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

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).[1]

	SD		TC	
Fatty Acid	Relative %	Crude Fat %	Relative %	Crude Fat %
14:0	1.36	1.04	3.27	2.35
14:1	0.07	0.05	0.10	0.07
15:0	0.17	0.13	0.29	0.21
15:1	0.10	0.08	0.08	0.06
16:0	17.90	13.59	18.24	13.11
16:1n 7	2.48	1.88	3.78	2.71
16:2	0.15	0.11	0.34	0.24
16:3	0.15	0.11	0.48	0.35
16:4			0.15	0.11
17:0	0.31	0.24	0.37	0.26
18:0	4.87	3.70	4.94	3.55
18:1n 9	30.25	22.97	22.26	16.00
18:1n 7	1.81	1.37	1.84	1.32
18:2 n 6	32.47	24.66	21.34	15.34
18:2 n 4			0.08	0.06
18:3 n 6	0.29	0.22	0.26	0.19
18:3 n 3	2.18	1.65	1.62	1.16
18:4 n 3	0.17	0.13	0.56	0.40
20:0	0.29	0.22	0.33	0.24
20:1 n 9	0.87	0.66	0.84	0.61
20:2 n 6	0.31	0.23	0.30	0.22
20:3 n 6	0.23	0.18	0.62	0.45
20:4 n 6	0.27	0.21	4.88	3.51
20:4 n 3	0.10	0.07	0.41	0.29
20:5 n 3	0.96	0.73	2.98	2.14
22:0	0.17	0.13	0.34	0.24
22:1 n 9	0.15	0.11	0.07	0.05
21:5 n 3			0.11	0.08
22:4 n 6			0.09	0.07
22:5 n 6			1.46	1.05
22:5 n 3	0.25	0.19	0.66	0.48
22:6 n 3	0.77	0.58	5.65	4.06
24:0	0.14	0.11	0.31	0.23
n/a	0.77	0.59	0.79	0.57
Total % n 3	4.42	3.36	11.99	8.62
Total % n 6	33.57	25.50	28.95	20.80
Ratio: n 3/ n 6	0.132	0.132	0.414	0.414

[1] analysis by New Jersey Feed Laboratory, Inc., Trenton, NJ

aeration. Total –ammonia-N, nitrite-N and pH parameters were measured twice a week. This data is summarized in Table 2.

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 was recorded and used to calculate relative weight (W_r). This condition index is determined by the equation:

$$W_r = (W / W_s) \times 100$$ (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:

$$\log_{10}(W_s) = -5.800 + 3.294 (\log_{10} TL)$$ (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 x 0.47 x 0.61 m in size containing 670-837 liters of water.

Hormone injections were administered in two doses; a priming injection of 30 µg/kg luteinizing hormone releasing hormone analog (LHRHa), followed 12 hours later by a resolving dose of 150 µg/kg. Hormone was purchased from American Peptide, Sunnyvale,

Table 2. Water quality: average measurement (min, max) of ponds, stocked with channel catfish female brood fish at ~1,322 kg/ha, per treatment over the 10 week culture period.[1]

Parameter	SD	F	TC
AM DO (mg/l)	6.88 (3.84, 9.94)	6.95 (3.73, 9.67)	6.91 (3.82, 9.50)
PM DO (mg/l)	8.37 (5.58, 11.19)	8.41 (5.11, 11.86)	8.25 (5.08, 10.39)
AM Temperature °C	19.71 (12.6, 26.3)	19.79 (13.0, 26.5)	19.53 (8.28, 26.4)
PM Temperature °C	23.48 (14.8, 31.3)	23.37 (15.1, 30.8)	23.46 (14.9, 30.7)
pH	7.79 (7.50, 8.20)	7.83 (7.50, 8.20)	7.79 (7.50, 8.10)
NH_3 (mg/l)[2]	0.28 (0.00, 0.86)	0.18 (0.00, 0.45)	0.32 (0.00, 1.31)
NO_2 (mg/l)	0.02 (0.00, 0.06)	0.02 (0.00,0.06)	0.02 (0.00, 0.06)

[1]Water samples for pH, NH_3 and NO_2 were collected at dawn. NH_3 and NO_2 levels determined by chemical methods adapted from Parsons et al. (1984)

CA. 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 anesthetized in buffered 150 mg/l tricaine methane sulfonate (MS-222) (Argent Chemical Laboratories, Redmond, WA). Females were then stripped and eggs were collected, in 50-150g 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^{-7} per 100 grams of eggs. To obtain the sperm, the males were sacrificed, 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 diluted with saline 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 minutes. When the egg had formed a cohesive mass, they were transferred to an egg basket that was placed with 8 other baskets in a 154 liter trough with ~6 liters per minute water exchange, two air stones and a slowly turning paddlewheel. 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. Oxygen was spot checked in the troughs

to assure that dissolved oxygen 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.

Paddlewheels were turned on after the youngest egg was at least 4 hours old. Formalin treatments of 100 mg/l began 12 hours after fertilization. After 24 hours, the eggs recieved three treatments per day alternating between 35 mg/l copper sulfate and 100 mg/l formalin. Just prior to hatch, copper treatments were withheld and the formalin treatment was reduced to 50 mg/l due to the increased vulnerability of embyros at this stage (Small and Chatakondi, 2006). Individual egg masses were weighed and visually assessed 24 hours pre-hatch to determine percent 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 (~7ml) that was frozen at -80°C for later biochemical analysis and a second (~30 eggs) that was stored in pre-weighed vials containing 5 ml of a 5% formalin solution.

Egg metrics

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/gram. A digital photograph was taken of each

sample and analyzed with Image Pro Express v. 4.5.1.3 (Media Cybernetics inc., Bethesda, MD). At least 15 eggs were then randomly selected and measured for a mean egg diameter per fish.

Biochemistry

For biochemical procedures, a portion of the egg sample (~1g) belonging to an individual fish was sub-sampled. These sub-samples 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. The lipid portion was then further analyzed qualitatively by gas chromatograph to determine composition. Methods for these procedures were adapted from Folch et al. (1957) and AOAC (1990). For lipid extraction, 0.2-0.5g 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 hours. 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.

These 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 chromatograph analysis, 0.1 µl aliquots were used. The samples were analyzed with a gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland; Model GC-17A) equipped with

a flame ionization detector and an Omegawax 530 capillary column (30 m x 0.53 mm; Supelco, Bellefonte, Pennsylvania). The initial temperature in the column was 140°C, two minutes after the initiation of the analysis the temperature was increased at a rate of 3°C/minute 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. Protein was analyzed by the micro- kjeldahl technique adapted from Ma and Zuazago (1942).

Data analysis

Data was 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 was nested by pond and analyzed by treatment. The Student–Neuman–Keuls multiple comparison test was used to determine significant differences among treatment means (Steel and Torrie, 1980). Spawning percentage, a binary response, was analyzed with logistical analyses. All statistical analyses were conducted using SAS (V9.1., SAS Institute, Cary, NC, USA).

III. RESULTS

Brood stock size and condition is presented in Table 3 for the three treatment groups: standard feed (SD), forage fish supplementation (F) and top-coated diet (TC). The size of fish and their associated condition index was similar among treatments both at stocking and at harvest. Brood stock performance parameters are presented in Table 4. Brood stock performance was normalized in two ways: 1) weight of fish that spawned, and 2) weight of fish that were harvested. The second value includes the weights of all the fish from the pond, regardless of if they spawned or not. These values, (with the exception of number of eggs/kg female that spawned) displayed a numerically increasing trend from SD through F to TC. Percent spawn increased from 65% to 77%, fry production increased by ~600 fry/kg brood fish, and percent 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 top coated diet ($p=0.003$) with an increase from 138.3 to 155.9 grams of eggs/kg female that spawned for the SD and TC treatments, respectively.

Egg characteristics are presented in Table 5. Eggs size, both in diameter and 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

Table 3. 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.

Parameter	SD	F	TC	p-value[1]	PSE[2]
Initial length (mm)	577.6	580.5	581.0	0.048 (0.809, 0.019)	23.459
Final length(mm)	612.2	613.7	615.2	0.024 (0.866, 0.009)	20.608
Initial weight (g)	2211.0	2231.0	2287.8	0.100 (0.528, 0.061)	296.108
Final weight (g)	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
Relative weight (W_r) final	114.5	112.8	113.7	0.636 (0.539, 0.561)	6.335

[1] Analysis was performed using ANOVA models. Where data for individual fish was available, data was 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.

[2] Pooled Standard Error

Table 4. Brood stock performance 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[1].

Parameter	SD	F	TC	p-value[2]	PSE[3]
Percent spawn[4]	65.2	74.5	77.3	0.533	-
No. eggs/kg female spawned	7435	7141	7266	0.510 (0.910, 0.354)	455.1
g eggs/kg female spawned	138.3[b]	135.3[b]	155.9[a]	0.003 (0.034, 0.010)	10.6
g eggs/total kg females harvested[5]	87.3	99.8	112.8	0.375	14.5
Total fry/kg females spawned	2,875	2,933	3,457	0.041 (0.197,0.046)	439.9
Total fry/kg females harvested	1,787	2,146	2,370	0.454	439.7
Percent viable pre-hatch fry	40.6	41.6	47.4	0.010 (0.270,0.008)	5.8
Total fry	291,117	376,067	433,583		

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on a Student Newman-Keuls multiple range test.

[2] Broodstock 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

[3] Analysis was performed using ANOVA models. Where data for individual fish was available, data was 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.

[4] Pooled Standard Error

[5] Percent spawn was analyzed as a binary response, using an exact logistic analysis.

[6] 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 5. 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 top coated with HUFAs (TC), in the spring prior to spawning.[1]

Egg characteristic	SD	F	TC	p-value[2]	PSE[3]
Mean egg weight (mg)	18.9[b]	19.1[b]	21.4[a]	0.001 (0.001,0.001)	0.01
Mean egg diameter (mm)	3.5[b]	3.6[b]	3.8 [a]	0.001 (0.001,0.001)	0.14
Mean % lipids	5.3	5.7	5.4	0.109	0.49
Mean % protein	16.4	16.5	15.9	0.08	0.58
Mean % moisture	74.4	74.7	74.7	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

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on a Student Newman-Keuls multiple range test.

[2] Analysis was performed using ANOVA models. Where data for individual fish was available, data was 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 alone and the pond (nested by treatment) alone, respectively.

[3] Pooled Standard Error

treatments had similar moisture, protein and lipid levels (74.4-74.7%, 15.9-16.4%, and 5.3-5.7% 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 6. The lipid molecules of interest (HUFAs and their precursors) are highlighted in table 7. The data indicates 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 figure 1.

The fatty acid profiles of the polar and neutral fractions of the egg lipids are presented in tables 8 and 9. The selected HUFAs and their precursor found in the polar fraction are highlighted in Table 10. 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 9). 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 are presented in figure 2. Increased proportions of EPA and DHA

Table 6. Mean percentage (± 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 top coated with HUFAs (TC) in the spring prior to spawning.[1]

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

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on Student Newman-Keuls multiple range test.

* C:19 was added as an indicator molecule for analysis by gas chromatograph.

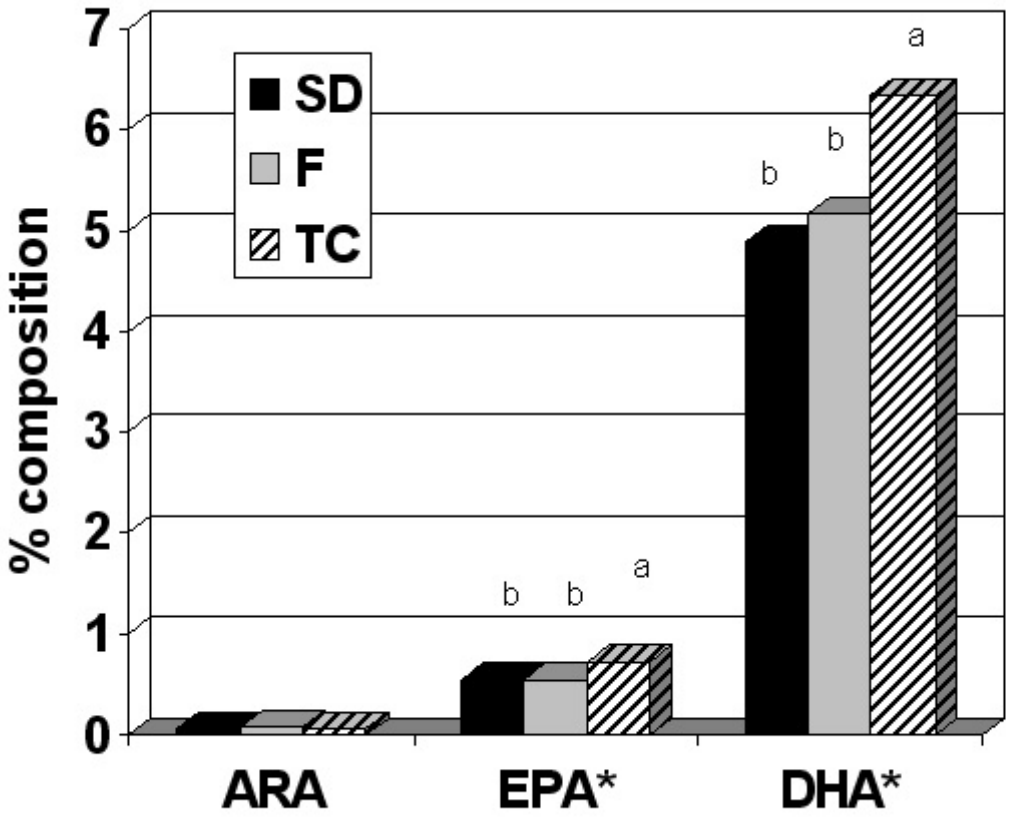
Table 7. Mean percentage of selected poly-unsaturated fatty acid content of channel catfish eggs fed three diets; a standard ration (SD), standard ration supplemented with forage fish (F) and a standard ration top coated with HUFAs (TC) in the spring prior to spawning.[1]

Fatty acid	SD	F	TC	p-value	PSE[2]
Linoleic acid (18:2n6)	8.931[b]	9.381[a]	8.831[b]	0.021	0.29
Linolenic acid (18:3n3)	0.491	0.495	0.499	0.962	0.04
ARA (20:4n6)	0.064	0.093	0.064	0.055	0.02
EPA (20:5n3)	0.545[b]	0.564[b]	0.730 [a]	0.001	0.03
DHA (22:6n3)	4.884[b]	5.171[b]	6.336 [a]	0.001	0.23
∑ n-6	14.609[b]	15.312[a]	13.599[c]	0.001	0.51
∑ n-3	13.478[b]	13.938 [ab]	14.611[a]	0.018	0.55
n-3/ n-6 ratio	0.922[b]	0.910[b]	1.074 [a]	0.001	0.02

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on Student Newman-Keuls multiple range test.

[2] Pooled Standard Error

Figure 1. Relative percent 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 top coated with HUFAs (TC) in the spring prior to spawning.



*Denotes variables that were found to be statistically significant ($p<0.05$) between treatments

Table 8. Mean percent 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 top coated with HUFAs (TC) in the spring prior to spawning.[1]

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[b]	0.61 ± 0.230[a]	0.56 ± 0.035[b]	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[b]	6.91 ± 0.406[a]	6.21 ± 0.430[b]	0.004
C19:0 *	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[a]	3.63 ± 0.484[a]	2.68 ± 0.264[b]	0.001
C22:5n3	1.87 ± 1.914	1.74 ± 0.709	1.15 ± 0.120	0.384
C22:6n3	8.10 ± 0.383[c]	9.13 ± 1.191[b]	11.08 ± 0.793[a]	0.001
∑ n-6	14.33 ± 0.705[a]	14.83 ± 1.076[a]	13.07 ± 0.920[b]	0.002
∑ n-3	23.18 ± 1.692[b]	23.12 ± 1.592[b]	25.98 ± 1.406[a]	0.001
n-3 / n-6	1.62 ± 0.179[b]	1.56 ±0.094[b]	1.99 ± 0.073[a]	0.001

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on Student Newman-Keuls multiple range test.

* C:19 was added as an indicator molecule for analysis by gas chromatograph.

Table 9. Mean percent 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.[1]

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[b]	3.24 ± 0.323[a]	3.47 ± 0.223[a]	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[b]	10.09 ± 0.973[b]	11.44 ± 1.042[a]	0.001
C19:0 *	16.09 ± 2.907[a]	13.99 ± 2.064[ab]	10.50 ± 7.042[b]	0.048
C18:3n-3	0.46 ± 0.078[b]	0.51 ± 0.068[ab]	0.60 ± 0.119[a]	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[b]	1.92 ± 0.172[a]	1.97 ± 0.169[a]	0.023
C20:3n-3	0.92 ± 0.078	1.04 ± 0.233	0.936 ± 0.119	0.232
C20:4n6[2]	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
∑ n-6	11.19 ± 0.694[b]	12.01 ± 1.129[b]	13.41 ± 1.209[a]	0.001
∑ n-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

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on Student Newman-Keuls multiple range test.

[2] ND = components that were below detectable limits

* C:19 was added as an indicator molecule for analysis by gas chromatograph.

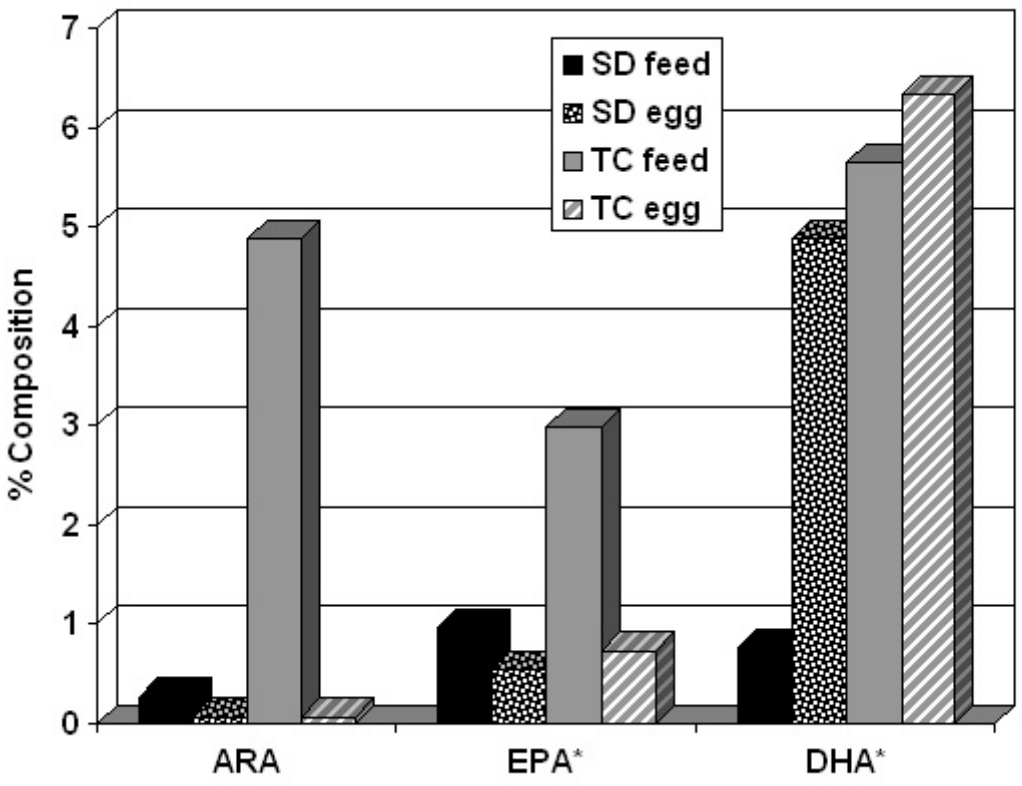
Table 10. Mean percentage of selected poly-unsaturated fatty acids in the polar fraction of egg lipids from female channel catfish fed three diets; a standard diet (SD), standard diet supplemented with forage fish (F) and a standard diet top coated with HUFAs (TC) in the spring prior to spawning.[1]

Fatty acid	SD	F	TC	p-value	PSE[2]
Linoleic acid (18:2n6)	6.321[b]	6.915[a]	6.212[b]	0.006	0.126
Linolenic acid (18:3n3)	0.284	0.291	0.268	0.811	0.032
ARA (20:4n6)	0.176	0.169	0.147	0.806	0.393
EPA (20:5n3)	0.793	1.076	1.198	0.151	0.157
DHA (22:6n3)	8.072[c]	9.134[b]	11.085[a]	0.001	4.955
∑ n-6	14.289[a]	14.831[a]	13.074[b]	0.003	0.256
∑ n-3	23.086[b]	23.121[b]	25.977[a]	0.01	0.611
n-3/ n-6 ratio	1.621[b]	1.562[b]	1.990[a]	0.001	0.055

[1] Means not sharing a common supercript within a row are significantly different ($p < 0.05$) based on Student Newman-Keuls multiple range test.

[2] Pooled Standard Error

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.



* HUFA molecules for which an increased availability in the diet correlated to an increased incorporation in the egg

in the diet were correlated to increased relative quantities of these molecule 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.

IV. 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 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 4 and 5). While ovodeposition in channel catfish may begin in fall (Brauhn and McCraren, 1975, Newman, 1990), the data presented here suggests that the lipid profile of the egg, at least, can still be affected by the diet of the fish within three 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 (Figure 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 7 and Figure 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 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 freshwater species, whose lipid metabolism is more complex. Gatlin and Stickney (1982) demonstrated that HUFA supplemented diets had little affect 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 triacylgycerols (TAGs) 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 signaling. The distinctly higher proportion of HUFAs in the polar fraction from the egg samples analyzed 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 ten. 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 9).

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 4). The same is true for the proximate composition of the eggs across treatments (Table 5). Where this data arises from individual fish measurements, the statistical analyses of these variables frequently indicates 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 3). 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 suggests 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 and Stickney, 1982, Ibeas et al., 1994, Francis et al., 2006). In the commercial management of catfish brood stock, fish are provided an optimum environment in which to mature, accumulate energy and ingredients and develop oocytes. The nutrition of these fish, however, typically remains unchanged or only sightly augmented (Kelly, 2004). A diet rich in PUFAs and low in HUFAs such as a commercial feed (PUFA : HUFA ratio in SD ≈14:1) is sufficient for growing catfish (Gatlin and Stickney, 1982), but it may be insufficient for the optimal maturation of channel catfish ovaries, as suggested by the trends seen here. In order 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 represented by the fish in the SD treatment whose HUFA complement of the eggs was dramatically increased compared to the diet (Figure 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, Torrans and 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 and Muller-Navarra, 1997, Oster, 2002, USDA, 2006). While this type of lipid supplementation has previously shown a marked improvement in egg and brooder quality (Torrans and Lowell, 2001), the affects of forage fish in this study were overshadowed by the diet with a direct supplementation of menhaden fish oil, arachidonic acid and docosahexaenoic acid.

Dietary lipids and their effect on egg quality is an understudied facet 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 involves pond spawning, reasonable hatch rates, and limited investment (Steeby and 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 if the return is ~600 or more fish per kg of female (Table 3). Additionally, this high cost feed appears to significantly affect 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 sub-optimal 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 top-coated diet significantly increased: 1) the HUFA complement of the egg (Tables 7 and 10, Figures 1 and 2), 2) the egg size, both weight and diameter (Table 5) and the total egg mass size per kg female brooder that spawned (Table 4). The addition of forage fish to the brood stock ponds at ~28 kg/ha had no significant effect compared to a commercial feed alone ($p<0.05$)(Tables 4-9) except in the DHA relative abundance in the polar fraction of the egg lipids (Table 10). The effects of both the F and TC diets upon many parameters such as percent 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 4 and 5)

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, Tvieten 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 Figure 2) but unlike other HUFA

molecules it appeared in the egg only in trace amounts (0.064%) (Table 6 and Figure 2). Similarly, the neutral fraction of the lipids from eggs in the TC treatment had significantly increased levels of n-6 fatty acids (Table 8). 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 a brood stock diet with an enriched lipid source has a positive effect on the size and quality of eggs from female channel catfish when used for hybrid production. Additionally, 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. In the production of hybrid catfish, the female brood fish management program should seek to provide an artificial diet with an increased HUFA complement in order 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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