Effects of Incubation Temperature and Parental Male Species on Hatching Success and Progeny Performance of Channel Catfish and Hybrid Catfish

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Abstract: Channel catfish (*Ictalurus punctatus)* x blue catfish (*I. furcatus)* hybrid fry production is variable and inconsistent in hatcheries, and there is sometimes an unsatisfactory reduction in the yield of viable fry that occurs during the final weeks of a spawning season. There are several possible reasons for these inconsistencies of production—this study investigates two: hatchery water temperature and the species of the parental male. Regarding water temperature, broodfish are often exposed to 30°–35° C temperature in ponds during the final weeks of spawning season in late spring, resulting in poor egg quality, hatching success, and fry survival. In this study, broodfish were held at optimal temperatures (26.6° C), and fertilized eggs were incubated at either 26.6° C or 32.2° C to approximate water temperatures of peak and latter part of the spawning season. To study effects of male species on spawning success, eight catfish females were induce-spawned and stripped eggs were fertilized with either channel catfish sperm to produce channel catfish families or blue catfish sperm to produce hybrid catfish families. Although incubation temperature and parental male species can affect hatching success of catfish eggs in the hatchery, subsequent survival and progeny performance in this study were unaffected under hatchery conditions. Results of this study suggest that optimizing incubation temperatures and broodfish selection during embryo development are essential for consistent and increased hybrid catfish fry production. Findings from this study can help catfish production by improving the efficiency of hatchery production and decreasing the cost per fish to produce in private or state hatcheries.

Key words: embryonic development, hormone-induced spawning, survival, growth

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The U.S. farm-raised catfish industry is transitioning from semi-intensive channel catfish (*Ictalurus punctatus)* production to intensive channel catfish x blue catfish (*I. furcatus)* hybrid catfish production. This is largely due to recent improvements in hatchery production of hybrid catfish, lower feed prices, higher survival, and better adaptability of hybrid catfish to intensive production systems. Presently, hybrid catfish account for more than 60% of catfish raised in the industry (Torrans and Ott 2018). Hybrid catfish are produced by hormone-induced spawning of channel catfish, stripped eggs are then fertilized with blue catfish sperm, and the fertilized eggs are incubated in hatcheries (Kristanto et al. 2009). Even though hybrid catfish fry production is increasing in hatcheries, inefficiencies still exist in hatchery production that need to be identified and addressed to improve production.

Gametogenesis and final maturation of channel catfish takes place in ponds, where broodfish are exposed to 24°–35° C waters during the spawning season (Davis et al. 1986, Arnold et al. 2013). The spawning season typically extends over 6 to 10 weeks from mid-April to June. Channel catfish spawn once a year, and spawning will cease or eggs will resorb if water temperatures deviate beyond the suitable range of 24°–30° C (Brauhn 1971), which can occur during passage of cold fronts in spring or unusually warm conditions during spring (Huner and Dupree 1984). The primary environmental variable influencing gonad development and spawning is temperature, which can be altered to manipulate the timing of reproduction (Kelly and Kolher 1996). However, any deviation from optimal incubation temperature results in significant reductions in hatching success and further egg survival is a function of both fertilization and embryo incubation conditions (Wipf and Barnes 2012).

Tolerance limits to abiotic stressors such as dissolved oxygen, temperature, and salinity are particularly narrow during early embryogenesis in fish (Geffen et al. 2006). Differences in paternally derived variation in thermal sensitivity at this critical period have been reported for some fish species (e.g., Dahlke et al. 2016). The combination of incubation temperature and parental male species influence on offspring survival and life-history traits is often complex and interactive in nature. Most commercial catfish hatcheries use a half-sibling (half-sib) family strategy to fertilize large quantities of stripped eggs, which involves using progeny derived from female parent sired by sperm pooled from two or more male individuals of a catfish species to overcome fertility problems (Barnes et al. 2003). Individual half-sibling family group also vary in their levels of response to their environment. Hence, it was hypothesized that incubation temperature and parental male species may influence hatching success and progeny performance of stripped channel catfish eggs. Incubation temperature has a direct effect on the timing of embryonic development and hatching success.

Prior studies have established thermal effects on developing eggs in broodfish and embryonic development in hatcheries that affect embryonic development, fitness, and hatching success (Salinas and Munch 2012, Jonsson and Jonsson 2014).

Water temperature is a critical factor that can influence early life history of fish through the thermal regimes experienced by parents as well as that of the developing progeny (Jonsson and Jonsson 2014). Knowledge of fish reproductive responses to temperature regimes is essential for hatchery operations whose objective is to optimize reproductive output and are reliant on information about parental and early embryonic rearing environments. Elevated temperatures during incubation in fish cause skeletal abnormalities (Dionisio et al. 2012), pericardial edema (Kurokawa et al. 2008), and poor swimming performance (Burgess et al. 2006). Also, an increase in incubation temperature leads to smaller larvae (Finstad and Jonsson, 2012), because of higher metabolic rate and energy consumption that increase exponentially above optimum temperature (Portner 2010).

The purpose of the study was to determine the influence of incubation temperature and parental male species effects on hatching success and subsequent progeny performance of channel catfish eggs. It is very difficult to mimic prolonged warm temperatures of ponds, so stripped eggs produced at optimal temperatures were exposed to two incubation temperatures of 26.6° C and 32.2° C to mimic peak and late spawning season water temperatures. Thus, the following objectives were assessed in this study: 1) influence of incubation temperatures (26.6° C vs 32.2° C) on hatching success of channel catfish and hybrid catfish; 2) effect of parental male species (channel catfish vs blue catfish male) on the hatching success of fertilized eggs; 3) percent survival of channel catfish and hybrid catfish at 0, 5, 25, and 50 days post-hatch, and 4) weight gain, feed intake, and feed conversion ratio (FCR) of channel catfish and hybrid catfish fingerlings in a growth trial.

Methods

This study was conducted at the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Warmwater Aquaculture Research Unit in Stoneville, Mississippi. A hatching trial was conducted to determine effects of incubation temperature and parental sire on hatching success, survival, and percent increase in body weight. This study was conducted by hormone-induced spawning four-year-old Delta Select strain channel catfish and then fertilizing the stripped eggs with either pooled Delta Select strain channel catfish sperm or D&B strain of blue catfish sperm to produce channel catfish or hybrid catfish half-sibling group families.

Hormone-induced Spawning of Catfish

Channel catfish were individually hand selected for optimal secondary sexual characteristics and stocked in an earthen pond at 1,000 kg-ha^{-1} during fall 2015. On 13 May 2016, channel catfish held in the pond were seined, and 25 gravid fish were individually selected from the pond and transported to the hatchery facility. Gravid catfish were placed in individual soft-mesh bags and suspended in a concrete raceway (water temperature = 26.6° C, pH = 8.6, and dissolved oxygen = 6.8 mgL^{-1}) following the methods described by Chatakondi (2014). Luteinizing hormone-releasing hormone analog (LHRHa, Syndel USA, Ferndale, Washington) was administered in two doses: a priming intraperitoneal injection of 20 μgkg^{-1} body weight followed by a resolving dose of 80 μgkg^{-1} body weight after 15 h (Kristanto et al. 2009). Ovulation response was checked every 3 h, beginning 26 h after the resolving dose of hormone injection. Once the signs of ovulation were observed, ovulating fish was anesthetized by immersion in 200 ppm of tricaine methanesulfonate. Eggs were hand-stripped into a 22.5-cm pan greased with a thin layer of vegetable shortening and weighed to the nearest 0.1 g. Testes were obtained from two male channel catfish to make pooled channel catfish sperm solution by macerating testes in 0.85% saline solutions. Testes from two blue catfish were used to make a similar pooled blue catfish sperm solution.

Fertilization, Incubation, and Hatching Success

Eggs from eight channel catfish females were stripped, gently mixed with a plastic spoon, and in-vitro pH of the ovulated eggs mixture was measured following the procedures described by Chatakondi and Torrans (2012) with an electrode using a HI9321 pH meter (Hanna Instruments, Ann Arbor, Michigan). Eight 5-g (approximately 250 eggs) were taken from each female catfish. Eggs held in four cups were fertilized with 0.5 mL of pooled channel catfish sperm to produce channel catfish half-sib group families and eggs held in the remaining four cups were fertilized with 0.5 mL of pooled blue catfish sperm to produce half-sib group hybrid catfish families. Two channel catfish and two hybrid catfish group families were incubated at 26.6° C while the remaining two channel catfish group families and two hybrid catfish families were incubated at 32.2° C. Eggs were incubated in a marked suspended 2-mm mesh basket (15 cm x 7.5 cm x 5 cm) in a 20-L polycarbonate aquarium which was a part of the "aquarium rack" system described in detail by Small (2006). Each rack system consisted of eight aquaria provided with flow-through water and diffused air with a common water-treatment system consisting of mechanical filtration, biofiltration, and temperature control. Two mesh baskets were suspended in each aquarium: two of the four rack systems were supplied with 26.6° C water of identical quality and the remaining two rack systems were supplied with 32.2° C water of identical quality. The water supply in the rack systems was supplemented with commercial grade calcium carbonate to attain 60 mgL^{-1} of calcium hardness and dissolved oxygen was maintained above 6.8 ppm. Water in rack systems were heated with immersion heaters (ELTA IIII-R14S-PL1, Process Technology, Willoughby, Ohio) to maintain the desired water temperature in each tank during incubation. Dissolved oxygen and water temperature were monitored three times daily with a model 58 electronic meter (Yellow Springs Instruments, Inc., Yellow Springs, Ohio) during incubation.

Percent neurulation was subjectively evaluated to confirm eggs were developing in all the hatching cups. Percent hatch was expressed as a proportion of the total number of live sac fry to a total number of eggs. After hatching, fry were gradually acclimated to 26.6° C in a marked 80-L flow-through aquaria (1$Lmin^{-1}$, dissolved oxygen= 6.6 mgL^{-1}). At 5 days post-hatch, 100 fry were counted and stocked in two marked 80-L aquaria. Fry were fed four times a day with 55% crude protein No. 1 starter feed (Rangen, Inc. Buhl, Idaho). Fry were fed twice daily from day 1–15 with automatic feeders. Fry were counted at the end of 25 days and 50 days to determine survival in aquaria. At day 50, fry density was reduced to 50 fish per aquaria and were fed once daily to satiation.

Growth Studies in Aquaria

Four-month-old fish (those which had been previously raised as 50-d-old fish) representing four of eight half-sibling group families of channel catfish and four of the eight half-sibling group families of hybrid catfish families (described previously) were selected for a six-week growth study in replicated 80-L aquaria. The number of families were reduced due to limited number of aquaria available for the growth study. A total of 16 families of fish (4 females x 2 genotypes x 2 incubation temperature) with three replicated aquaria per family (15 fish per aquarium) were used in this study. Ten fish were randomly chosen from each aquarium to obtain initial weight (g) and length (TL, mm) of individual fish before the start of the growth study. Fish in all aquaria were fed once daily with a 35% protein 3-mm commercial fingerling feed (Fish Belt Feeds, Morehead, Mississippi) to satiation from a pre-weighed container designated for each aquarium. At the end of the study, fish from an individual aquarium were weighed as a group and 10 fish were randomly measured for individual body weight (g) and TL (mm) and Feed conversion ratio (FCR) for each aquaria was calculated as FCR = feed fed (g) / weight gain (g).

Statistical Analyses

Prior to analyses, percent data were arc-sine root transformed to meet the normality assumptions of parametric tests. Four periods of survival were examined at 0 (hatch), 5, 25, and 50 days. Data were analyzed via two-way ANOVA, using Proc Mixed (SAS Institute 2012). The statistical model included incubation temperature and sire as fixed effects for all the analyses. Because the interaction of female and sire was not significant, data from individual females were pooled. Pooled SE was presented in the tables because variances of means were approximately equal. The variation among female x sire x incubation temperature in a replicated basket was used as an experimental error to tests of significance set as P =0.05. Variables analyzed include percent hatch, percent survival and percent increase in weight gain, mean feed intake and FCR to assess the effects of incubation temperature and paternal male on stripped channel catfish eggs.

Table 1. Mean survival at hatch (0 d), 5 d, 25 d, and 50 d of hybrid catfish and channel catfish incubated at two temperatures (26.6° C and 32.2° C). Means with different subscripts vary ($P < 0.05$).

			Survival (%)		
Species	Temperature (°C)	Hatch %	5-day	25-day	50-day
Hybrid	26.6	49.6^a	90.2^a	83.4^a	73.5^a
Channel	26.6	44.0^b	87.4^a	78.4^a	68.6^a
Hybrid	32.2	43.7^b	86.0^a	74.8^a	65.7^a
Channel	32.2	35.7^c	84.2^a	73.2^a	58.9^a

Results

Eggs incubated at 26.6° C hatched approximately in five days (120 h), and eggs incubated at 32.2° C embryos hatched approximately in four days (96 h). At 0 days, mean percent survival (percent hatching success) of hybrid catfish (49.6%) was higher than mean percent survival of channel catfish (44.0%) at 26.6° C, and mean percent survival of hybrid catfish (43.7%) was higher than mean percent survival of channel catfish (35.7%) (F = 4.32, df = 7, 32; P = 0.02). At five days, mean percent survival of hybrid catfish fry was similar to mean percent survival of channel catfish fry at 26.6° C and 32.2° C (F = 0.59, df = 7, 32; P = 0.76). At 25 days, mean percent survival of hybrid catfish eggs was similar to channel catfish fry at 26.6° C and 32.2° C (F = 1.50, df = 7, 32; P = 0.20). At 50 days, mean percent survival of hybrid catfish fry was similar to mean percent survival of channel catfish fry at 26.6° C and 32.2° C (F = 1.15, df = 7, 32; P = 0.35) (Table 1).

Mean initial weights of four-month-old fingerlings representing half-sib channel catfish and hybrid catfish families incubated at 26.6° C or 32.2° C that were evaluated for a six-week growth study in 80-L aquaria did not differ (F = 0.43, df = 1, 44; P = 0.51). Similarly, mean final weight of channel catfish and hybrid did not

Table 2. Effect of parental male (P) and incubation temperature (T) on final weight, percent weigh gain, feed intake, and FCR for four-month old fish in a six-week growth study. Means did not differ (*P > 0.05*) between different groups.

Species	Temperature (°C)	Initial weight (g)	Final weight (g)	Feed intake (g)	FCR
Channel	26.6	10.43	23.2	182.3	0.91
Channel	32.2	10.91	24.3	188.9	0.95
Hybrid	26.6	8.90	21.0	175.6	0.97
Hybrid	32.2	8.96	20.2	160.0	0.98

Table 3. Effect of two incubation temperatures and catfish species produced on hatching success (Mean ± SD) produced from eggs stripped from eight individual channel catfish. Means with different subscripts vary (*P < 0.05*) among females.

	26.6° C		32.2° C	
Female	Channel catfish	Hybrid catfish	Channel catfish	Hybrid catfish
1	$42.82^a \pm 2.57$	$38.59^a \pm 1.54$	$24.13^a \pm 2.85$	41.55b ± 2.19
10	$45.63^a \pm 3.61$	$55.32^c \pm 7.52$	$36.54^a \pm 5.34$	$44.58^b \pm 4.12$
11	$42.82^b \pm 2.57$	$40.23^b \pm 2.03$	$33.52^a \pm 2.80$	$42.41^b \pm 2.95$
12	$47.61^b \pm 2.30$	$58.20^c \pm 7.64$	$35.76^a \pm 3.86$	$45.61^b \pm 2.20$
13	$43.83^b \pm 3.04$	$48.54^b \pm 7.95$	$31.17^a \pm 0.93$	$45.15^b \pm 2.36$
15	$36.32^b \pm 5.90$	$40.97^b \pm 7.81$	$28.72^a \pm 1.80$	$34.25^a \pm 2.47$
2	$45.27^b \pm 4.10$	$53.61^c \pm 7.07$	$43.65^b \pm 3.74$	$48.11^b \pm 2.66$
9	$47.92^a \pm 2.30$	$61.04^b \pm 5.90$	$42.17^a \pm 3.49$	$58.03^b \pm 3.71$

differ ($F = 10.73$, df = 1, 44; $P = 0.37$). Mean feed consumed of channel catfish and hybrid catfish representing these four families did not differ ($F = 0.87$, df = 1, 44; $P = 0.35$). Mean FCR of channel catfish and hybrid catfish families did not differ ($F = 0.006$, df = 1, 44; $P = 0.79$). Mean percent weight gain of hybrid catfish and channel catfish in four families did not differ in the growth study ($F = 2.12$, df = 1, 32; $P = 0.07$) (Table 2).

Mean hatching success of hybrid catfish eggs and channel catfish eggs incubated at 26.6° C or 32.2° C from stripped eggs from eight individual channel catfish females varied among half-sib group families. At 26.6° C, mean hatching success of half-sib group of hybrid catfish families ranged from 38.6% to 61.0% and half-sib group families of channel catfish families ranged from 36.3%–47.9% ($F = 44.6$, df = 1, 32; $P = 0.01$). At 32.2° C, mean hatching success of half-sib group of hybrid catfish families ranged from 35.7%–58.0% and half-sib group families of channel catfish families ranged from 36.3% to 47.9% ($F = 40.8$, df = 1, 32; $P = 0.01$) (Table 3).

Discussion

Temperature exerts a major effect on all physiological processes of catfishes, and effects are generally more pronounced in developing embryos (Rambough 1996). Mean hatching success of hybrid catfish was higher than that of channel catfish at both incubation temperatures. Overall, survival at hatching success of hybrid catfish at 26.6° C was higher than all other species-temperature combinations.

In the present study, parental male species influenced the hatching success at both the incubation temperatures. Previously, Janhunen et al. (2010) evaluated parental male effects on Arctic charr (*Salvelinus alpinus*) embryonic viability and growth at 2° and 7° C incubation temperatures. Higher temperature resulted in higher mortality rates and less advanced development at hatching, and survival was attributed to maternal effects at low temperatures. Their findings suggested that thermal stress during incubation can modify early developmental traits and adaptive potential for environmentally induced variation of performance traits to corroborate with the findings of this study. Burt et al. (2012) evaluated five paternally and five maternally linked families of sockeye salmon (*Onchorhynchus nerka*) incubated at 12°, 14°, and 16° C from fertilization to hatch. Similar to the results of this study, influence of parental male was evident on offspring exposed to higher thermal regimes and was essential for spawning success and adaptation to environmental change. Channel catfish eggs sired by blue catfish sperm resulted in significantly higher hatching success at both the incubation temperatures.

Incubation temperature and parental male species effects on subsequent progeny performance were likely minimized in the present study due to optimal rearing and exogenous feeding under controlled aquaria conditions. Contrary to my findings, Heath et al. (1993) found significant sire and incubation temperature effects on relative growth and final wet weights of Chinook salmon (*Oncorhynchus tshawytscha*) incubated at two temperatures (8° and 10° C). Favorable incubation temperature conditions during egg development increased developmental rates and provided Chinook salmon a head-start relative to those developed under less favorable conditions.

Previously, Dunham et al. (1999) reported similar (55%–66.2%) fertilizing success with stripped eggs from 28 channel catfish fertilized either with channel catfish or blue catfish sperm. In the past 15 years, several improvements were made in assisted reproductive technologies in channel catfish (e.g., ovulating hormones, fertilizing protocols, broodfish husbandry) and may have likely led to the higher hatching success of hybrid catfish families compared to channel catfish families observed in the present study. Hatching success of channel catfish eggs sired by blue catfish sperm tended to be higher than those sired by channel catfish sperm in other studies (unpublished data, Jubilee Farms, Indianola, Mississippi). Heterotic effects of improved growth, survival and tolerance to stressors have been previously documented for hybrid catfish (Dunham and Smitherman 1987), resulting in widespread adoption in the U.S. farm-raised catfish industry. In the present study, higher hatching success of hybrid catfish may also be a trait likely attributed to heterotic effect; however, hatching success of individual families varied among channel catfish females emphasizing the need to select high quality broodfish for hybrid catfish fry production.

Hybrid catfish constitute approximately 60% of the catfish production in the catfish industry because hybrid catfish have higher growth rate, survival, and tolerance to common stressors and adaptable to intensive production systems (Chatakondi et al. 2018). Hybrid catfish fry are reliably produced by hormone-induced spawning of channel catfish in hatcheries, but their production is variable, inconsistent, and causes extensive disruptions in fingerling production. A focus of my research has been to investigate methods to consistently increase hybrid catfish fry production in hatcheries. In this study, I found that incubation temperature positively influenced the length of the fry as well as the hatching success of catfish eggs. Results from this study will help to formulate strategies to develop methods to hold broodfish in ponds, sire ovulated eggs, and incubate fertilized eggs at optimal temperature for a reliable and increased fry production in hatcheries.

Channel catfish have evolved to spawn during spring, but as water temperature increases in summer months, egg quality degrades and eggs eventually are resorbed. In the present study, incubating fertilized eggs at 32.2° C increased the pace of embryonic development but resulted in lower hatching success. Possible strategies to overcome exposure to higher temperatures during embryogenesis would be holding broodfish in deeper ponds or flushing broodfish ponds with well water to reduce summer water temperatures. Other strategies to consider may include out of season spawning of catfish (Brauhn 1971, Lang et al. 2003), environmental manipulation to simulate seasonal effects (Kelly and Kohler 1996), or genetic selection for early spawning of channel catfish.

This research will contribute to improving hybrid catfish aquaculture production but has wide implications for all hatchery-reared fish species. Determining how maternal and paternal components influence important developmental traits like hatch rate, survival, and progeny performance during early life stages will improve incubation techniques and broodfish selection. Many state and fish agencies rarely use artificial spawning techniques but do stock catfish brooders in ponds that are invariably exposed to warmer pond conditions that will hinder fingerling production. There is an increased demand for stocking aquacultured fish, especially hybrid catfish in public and private waterways. Findings of this study are relevant to hybrid catfish hatcheries involved with commercial aquaculture and several state and game agencies that produce catfish fingerlings in hatcheries to stock in public and private waterways (Munger et al. 2016). Reliable and continuous supply of gravid channel catfish raised in ponds is essential for hybrid catfish fry production in hatcheries. Spawning of catfish broodfish, embryo development, survival of embryos occurs within a narrow range of water temperature. Findings from this study illustrate how water temperature affects development of gravid female catfish and early stages of developing embryos. Hatchery managers can use this information to optimize broodfish holding conditions and hatchery incubation conditions. Improved hatching efficiency will reduce the cost of producing fish for stocking programs, resulting in higher stocking of public and private water bodies resulting in higher angler participation.

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