Effects of Exposure to Calcium-deficient Water on Fertilization and Hatching of Channel Catfish x Blue Catfish F_1 Hybrids

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Abstract: Most hybrid catfish are produced by fertilizing eggs from hormone-induced, strippable channel catfish (*Ictalurus punctatus*) females with sperm from blue catfish (*I. furcatus*). Water to most hatcheries is supplied from 300 to 400-m deep aquifer, yielding geothermal water of 25–30 C with low level of calcium hardness and hence supplemented with an external source of calcium. Many catfish hatchery water sources have low calcium concentrations and are supplemented with an external source of calcium. Nevertheless, failure of calcium pump or delivery system in commercial catfish hatcheries is not uncommon. This study examined 12 sequential 8-hour periods of exposure of hybrid catfish eggs to calcium-deficient waters from fertilization to hatch. Periodic exposure to calcium-deficient waters did not affect fertilization of hybrid catfish eggs. However, any exposure period of hybrid catfish eggs to calcium-deficient waters (0.4 mg/L) within 48 h post-fertilization reduced hatching success of hybrid catfish eggs compared to a control group not exposed to calcium-deficient waters. This 48-hour post-fertilization period appeared to be a calcium-critical period in hybrid embryo development. Frequent monitoring of calcium hardness in hatchery waters is critical to minimize losses in hybrid catfish fry production, especially during the first 48 h post-fertilization.

Key words: Calcium supplement, hatchery water, hatching success, critical period

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Even though channel catfish (*Ictalurus punctatus*) production has decreased over the last nine years, it remains the single largest aquaculture fishery in the United States. In 2011, approximately 335 million pounds of catfish were processed, a reduction of over 50% from the production in 2003 (National Agricultural Statistics Service 2012). Innovative approaches are needed in the catfish industry to reduce production costs and improve profitability in catfish farming. Adopting hybrid catfish (channel catfish female × blue catfish, [I. *furcatus*] male) in production ponds may be an avenue to improve productivity immediately. Raising hybrid catfish in production ponds enable the farmer to use the improved growth rate, survival, and feed conversion of these fish over the parental species to lower production costs (Chatakondi 2012). However, production of hybrid catfish involves induced spawning of channel catfish and fertilization with blue catfish sperm. Hatching efficiency is generally lower in hybrid catfish hatcheries compared to hatcheries using pond-spawned channel catfish eggs (Dunham et al. 2000). Important production parameters have been identified and a few have been addressed to improve the efficiency of hatchery production of hybrid catfish fry in commercial hatcheries (Chatakondi et al. 2011).

In hatcheries with soft water, calcium (in the form of calcium chloride) can be supplemented in the water supply through a peristaltic pump to achieve desired concentration of calcium (Yeager 1994). Therefore, hatchery systems can be adjusted to provide optimal water hardness levels for the incubation and hatching of different fish species (Chatakondi and Torrans 2012). Calcium hardness is related to egg swelling, which provides physical protection and room for the developing embryo, and egg and larval survival (Maetz 1974). Egg swelling occurs when the eggs draws in extracellular water because of greater osmotic pressure (Alderdice 1988), and the amount of swelling depends on the difference between the osmotic concentration of the perivitelline space and the extracellular water. An increase in egg swelling is observed with a decrease in water hardness or osmotic concentration of the incubating media (Rees and Harrell 1990).

Calcium hardness requirements for strip-spawned channel catfish eggs are higher than naturally-spawned eggs. Strip-spawned eggs are generally lower quality because of hormone treatment, handling stress, younger age, varying maturity stages, and possible mechanical damage suffered during the stripping process (Chatakondi and Torrans 2012). Soft water reduces egg turgor and increases the susceptibility of a developing egg to mechanical injury and decreased survival (Ketola et al. 1988). Additional handling of eggs during fertilization, water hardening, incubation in hatching troughs and chemotherapeutic treatments may all lead to significant mortalities in developing embryos (Krise 2001). Small et al. (2004) identified the calcium-critical period of naturally spawned channel catfish embryos to be the first 24 hours post-fertilization.

Mississippi hatcheries are supplied with groundwater from deep aquifers (242–454 m) in order to provide the proper water temperature for egg incubation. However, these waters are natu-

rally soft and have very low to non-existent calcium concentrations (Tucker and Steeby 1993). Hence, supplemental calcium must be added to hatchery waters for successful catfish production. In commercial hybrid catfish hatcheries, calcium concentration of hatching waters is monitored at least once daily in addition to routine measurements of temperature and dissolved oxygen (Jeff Baxter, Baxter Land Company, personal communication). As calcium is typically dispensed through a pump, failure of the pump or plugging of the delivery hose is routinely observed in hatcheries (Small et al. 2004) and the absence of calcium in waters may not be noticed for >8 h. The time of hybrid catfish embryo development from fertilization to hatch is approximately 90–96 h at incubation temperatures of 26–28 C (Chatakondi et al. 2011). Therefore, it was hypothesized that any 8-hour exposure period of post-fertilized hybrid catfish eggs to calcium deficient waters during embryogenesis may affect hatching success. The objective of the present study was to determine the extent of hatching losses in replicated baskets of hybrid catfish eggs exposed to sequential 8-h exposure periods to calcium-deficient waters during embryogenesis.

Methods

Mature female channel catfish (age 4) of 'Delta' select strain, currently being developed at the USDA Catfish Genetics Research Unit, Stoneville, Mississippi, were used in this study. Channel catfish with superior secondary sexual characteristics as described by Phelps et al. (2011) were selected and stocked at 1500 kg/ha in a freshly filled 0.4-ha earthen pond at the USDA Catfish Genetics Research Unit in March 2011. In June 2011, 10 gravid females were hand selected and placed in individual soft mesh bags suspended in a 10,000-L concrete tank supplied with flow-through water and compressed air (water temperature = 27.5 C, pH 8.6 and dissolved oxygen > 6.0 mg L^{-1}. Luteinizing hormone releasing hormone analog (LHRHa, Western Chemicals, Seattle, Washington) was administered in two doses, a priming intraperitoneal injection of 20µg kg^{-1} of body weight (BW) followed 15 h later by a 80µg LHRHa kg^{-1} BW following the protocols described by Chatakondi et al. (2011). Sperm was obtained from pond-raised D&B-strain blue catfish (age 6) by exercising and macerating the testes.

Ovulation occurred approximately 26–36 h after administration of the resolving dose. The mesh bags were slightly lifted above the water and examined for the presence of expressed eggs adhered to the bag. All 10 females expressed eggs; approximately 100–600 g of eggs were stripped from the females. To reduce variability in egg quality, only the female from which the most eggs were stripped was used in the study. The ovulating female was anaesthetized by immersion in a buffered tricane methanesulfonate solution (MS222; Argent Laboratories, Inc., Redmond, Washington), rinsed in clean well water, and dried with a towel; eggs were then hand stripped into a dried aluminum pan coated with a thin layer of vegetable shortening. Hand stripping was terminated when the eggs stopped flowing. Blue catfish sperm solution was prepared as per the procedures described by Kristanto et al. (2009). Six hundred g of stripped eggs were weighed in a greased bowl and 6 mL of blue catfish sperm solution was mixed with a plastic spoon. Approximately 500–600 eggs, determined by weight, were aliquoted to each of 39 greased cups. The eggs and sperm were activated with hatchery water (60 mg/L of calcium hardness) for 2 minutes, excess sperm and debris were washed off, and individual eggs water hardened for 10 minutes to form a spawn (group of individual eggs in an adhesive matrix).

Each spawn was placed in a fine mesh basket (7.5 × 7.5 × 15 cm) suspended in a 20-L aquarium housed in an aquaria rack system (Small 2006). Each aquarium was provided with recirculated water with 60 mg L^{-1} of calcium hardness at 26.1 C, compressed air to provide sufficient supply of oxygen, and a rear-side drain with a removable mesh screen. Each tank was independently supplied with water and drained at a constant rate of 7.5 L min^{-1} via an adjustable flow regulator. The reservoir tank was a 100-L plastic tank on the bottom rack with a submersible pump to circulate water. Twelve sequential 8-h intervals were chosen to determine the calcium critical period during hybrid catfish embryo development. Of the 39 spawns produced in this study, three spawns were randomly assigned for control treatment (not exposed to calcium deficient waters during embryogenesis), and the remaining 36 spawns were randomly assigned to one of the 12 sequential 8-h exposure periods to calcium-deficient waters during embryogenesis. Hatching of hybrid eggs was expected to occur on the fourth day (Chatakondi et al. 2011), hence the experiment was terminated after 96 h.

Each aquaria rack system was independent and water was maintained at 60 mg L^{-1} of calcium hardness by adding 1.3 mL of commercial grade calcium chloride (Aquacenter, Leland, Mississippi) to the well water for each 1 mg L^{-1} of calcium hardness in a recirculatory system. In the fourth aquaria rack system, three aquaria were maintained with unaugmented well water (0.4 mg L^{-1} calcium hardness). At 8-h intervals, a set of eggs (3 individual baskets with fertilized eggs) were transferred to the calcium-deficient system and incubated for an 8-h period. At the end of this period, baskets containing the eggs were transferred back to their previous aquaria until the completion of hatching process. Thus, by the end of the study, sets of incubating eggs had been exposed to calcium-deficient waters for 8 h from 0–8 h to 88–96 h post-fertilization.

Calcium hardness in each rack system was measured twice daily with a Hach Test kit model FF-2 with digital titration (Hach Chemical Company, Loveland, Colorado). Temperature and dis-

solved oxygen (DO) were measured by a YSI model-58 DO meter (YSI, Yellow Springs, Ohio). Ammonia, nitrite, and nitrate were measured using water quality test kits (Hach Chemical Company, Colorado). No antifungal treatment was applied to the recirculatory system because accumulation of these treatments has been shown to affect the embryonic development (Martins et al. 2009). Percent fertilization was expressed as a proportion of the total number of live eggs to the total number in a sample 24-h post-fertilization. Disassociation of the chorion was an indicator of an unfertilized egg. Percent hatch was expressed as a proportion of the total number of fry to the total number of fertilized eggs in the basket (Chatakondi and Torrans 2012).

Statistical analyses were conducted using the mixed model procedures of the Statistical Analysis System version 9.1 (SAS Institute Inc., Cary, North Carolina). Differences in mean percent fertilization and percent hatch among treatments were analyzed using one-way analyses of variance. Percent data were arcsine transformed to equalize the variance prior to the analysis. If differences were significant, means were separated by Tukey's *ad hoc* test. In all statistical comparisons, responses were considered significant at $P \leq 0.05$).

Results

All the water quality parameters measured were within the normal range during the conduct of the study. Percent fertilization of hybrid catfish eggs subjected to periods of calcium-deficient waters during embryogenesis did not differ among the treatments (Table 1). The group that was not exposed to calcium-deficient waters experienced the highest hatch of hybrid catfish eggs. However, mean percent hatch of hybrid catfish eggs exposed to any 8-h period of calcium-deficient waters prior to 48-h post-fertilization was 45%–61% lower (mean 56%) than the control group ($F = 17.58$, $df = 12, 26$ $P \leq 0.05$). In contrast, mean percent hatch of eggs exposed to any sequential 8-h periods to calcium-deficient waters after 48 h post-fertilization was similar to that of the control group ($P \geq 0.05$), on average only 14% lower (Table 1).

Table 1. Percent fertilization and hatch (mean ± SE) of channel catfish × blue hybrid eggs exposed to periodical 8-hour exposure to calcium-deficient waters (0.4 mg/L calcium hardness) from 0- to 96-h post-fertilization. Means within columns followed by the same letter were similar (Tukey's test, $P > 0.05$).

Period of exposure (Post-fertilization h)	Percent fertilization (mean ± SE)	Percent hatch (mean ± SE)
0	88.3 ± 6.6a	31.4 ± 1.7b
0–8	85.5 ± 5.8a	12.5 ± 1.74a
8–16	90.6 ± 3.3a	13.0 ± 1.59a
16–24	87.7 ± 7.8a	12.3 ± 2.37a
24–32	93.4 ± 5.1a	17.2 ± 0.9a
32–40	89.9 ± 6.8a	12.7 ± 2.01a
40–48	93.1 ± 6.8a	16.1 ± 0.9a
48–56	89.8 ± 5.2a	26.2 ± 1.6b
56–64	94.0 ± 3.2a	28.3 ± 1.45b
64–72	88.8 ± 6.6a	27.7 ± 1.29b
72–80	80.2 ± 9.4a	26.9 ± 2.33b
80–88	86.4 ± 8.2a	26.3 ± 0.4b
88–96	85.2 ± 8.8a	25.5 ± 3.2b

Discussion

Temperature and DO are regarded as the primary essential water quality parameters during the embryonic development of fish in hatcheries. However, calcium, which contributes to water hardness and total osmotic concentration, is also a key factor in egg and larval development of teleosts. The pioneering work of Tucker and Steeby (1993) led to the practice of supplementing calcium hardness in hatcheries to incubate naturally-spawned fertilized channel catfish eggs. Small et al. (2004) exposed naturally-spawned channel catfish eggs to low calcium waters at sequential 24-h intervals during embryogenesis to identify the calcium-critical period of naturally-spawned channel catfish eggs. The focus of catfish industry is shifting towards raising hybrid catfish in production ponds (Chatakondi and Torrans 2012), and hormone-induced spawning and artificial fertilization are currently practiced at several catfish hatcheries. Optimal calcium hardness to incubate hybrid catfish eggs was determined to be 75 mg L^{-1} of calcium carbonate, three times higher than the needs of naturally-spawned channel catfish (Chatakondi and Torrans 2012). The optimal concentration of eggs was further optimized to 60 mg L^{-1} of calcium carbonate (N. Chatakondi, unpublished data) to incubate hybrid catfish eggs.

In this study, hybrid catfish embryos were subjected to calcium-deficient waters during sequential 8-h intervals to determine the critical period for calcium in hybrid catfish embryos. Critical developmental stage of channel × blue hybrid embryo were identified previously to be 40 to 48 h (N. Chatakondi, unpublished data) and chemical treatments have been recommended to be withheld during this critical period of hybrid embryo development (SRAC 2005). Small et al. (2004) determined the calcium-critical period to be 24 h post-fertilization for naturally-spawned channel catfish eggs collected from ponds. However, spawned eggs collected from ponds are at least 12 to 24 h post-fertilization in age because the spawning process takes 4–6 h for a channel catfish female to deposit all her eggs (Clemens and Sneed 1957). Also, eggs are usually collected 8–18 h after spawning according to usual hatchery protocols. Therefore, the calcium critical period assessed in naturally spawned channel catfish would actually be 40–46 h post-fertilization, which was similar to the findings in this study.

In this study, as noted, eggs from a single female were used in all

the treatment periods; however, mixing eggs from different females likely would not have altered the outcome of the study. To the contrary, the high quality of eggs used in the study may have played a role in the hatching success of hybrid catfish eggs. Future studies should be conducted to assess the effect of calcium-deficient waters on the hatching success of varying qualities of stripped channel catfish eggs, which may be more widely applicable in hatchery settings where egg quality are not be closely monitored.

This study mimicked commercial hatchery conditions where calcium pumps often malfunction, resulting in loss of supplemental calcium additions to the hatchery waters for a period of time. Exposure of developing hybrid catfish eggs to calcium-deficient waters during any 8-h period up to 48 h post-fertilization significantly affected hatching success. Measures to overcome calcium supplement problems in hatching waters are known and can be easily performed. Results from this study open new possibilities in the management practices of commercial hybrid catfish hatcheries. Hatchery losses can be reduced by ensuring optimal levels of calcium hardness through heightened vigilance of hatching waters during the critical period of hybrid embryo development.

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