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Identification of a Calcium-Critical Period During Channel Catfish Embryo Development

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The United States farm-raised catfish industry produces approximately 1.6 billion channel catfish *Ictalurus punctatus* fry annually, generating over U.S. $24 million in income for commercial hatcheries (NASS 2002). Channel catfish embryo survival varies greatly in commercial hatcheries, and can range from 0–100% among egg masses collected from a single pond. Many factors, including temperature, water quality, disease, and management practices can have significant effects on channel catfish embryo development and survival (Weirich and Tiersch 1997; Small and Bates 2001; Small and Wolters 2003).

The aquifer used for channel catfish hatcheries in much of the Mississippi delta has a calcium hardness (Ca-hardness) of less than 10 mg/L as $CaCO_3$. Ca-hardness less than 10 mg/L has been shown to reduce survival and growth of sac- and swim-up fry (Tucker and Steeby 1993). As a result of their research, Tucker and Steeby (1993) recommended a minimum Ca-hardness of 10 mg/L as $CaCO_3$ for channel catfish hatchery water; however, the effect on survival from fertilization to hatch is unclear.

Calcium critical stages of embryogenesis have not been previously described for channel catfish. Although few would argue the importance of iono- and osmoregulation in developing fish embryos, little is known about the mechanisms of calcium balance in embryos. It is clear that freshwater teleosts tightly maintain plasma calcium concentrations (2–4 mM) despite potential exposure to a wide range of environmental calcium levels (Chou et al. 2002), and it is assumed calcium homeostasis is maintained in the developing embryo. Many iono- and osmoregulatory processes have been defined in post-larval fish (Perry and Wood 1985; Flik et al. 1986; McCormick et al. 1992; Patrick et al. 1997); however, there is some debate regarding calcium uptake in embryonic fish.

Research on calcium transport during development of embryonic rainbow trout demonstrates active calcium ion uptake during embryogenesis (Barrett et al. 2001); however, little research has been conducted to examine the effects of changes in external calcium concentrations on developing embryos and their survival. This study examined the effect of environmental Ca-hardness concentration less than 10 mg/L as $CaCO_3$ during sequential 24-h periods of channel catfish embryogenesis on survival to hatch.

Materials and Methods

Spawning and Egg Collection

Channel catfish brood fish were stocked into a 0.1-acre pond in Stoneville, Missis-

sippi, USA at a stocking density of 20 females and 10 males. Eight spawning containers were placed in the pond, and fish were allowed to spawn naturally. Spawning containers were checked daily during July 2002 for recently fertilized egg masses. Pond water analyses were conducted for pH, total hardness, Ca-hardness, and total alkalinity according to the methods of Boyd and Tucker (1992). Following removal from the pond, the eggs were treated with an iodine solution (Aquadine, Aquacenter, Leland, Mississippi, USA) by immersion at a dose of 100 ppm for 10 min. Stage of embryo development was then determined microscopically according to Makeeva and Emel'yanova (1993). A single egg mass of 380 g in the early blastodisc stage (less than 1-h post-fertilization) was selected, and subdivided into 24 approximately equal masses. The number of eggs per mass was calculated after determining eggs per gram.

Egg Incubation

The subdivided egg masses were randomly assigned to 24 76-L glass aquaria equipped with a wire hatching basket and an airstone. Twenty-one aquaria were allocated to treatments such that every 24-h developmental period post-fertilization to hatch was represented in triplicate. Time to hatch was expected to be 6–7 d (Small and Bates 2001). Egg masses in the three remaining aquaria received calcium-supplemented water throughout embryogenesis. Each aquaria was supplied with flowing well water (temperature, 26 C; pH, 8.6; alkalinity, 410 mg/L) at a rate of 7.6 L/min. Water circulation was maintained through vigorous aeration. Calcium chloride was supplied to the water via a chemical metering pump to achieve a Ca-hardness of approximately 100 mg/L as $CaCO_3$. A separate water manifold was utilized for supplying calcium-deficient water (< 10 mg/L Ca-hardness). The calcium-deficient water manifold was supplied by the same well, but bypassing the chemical metering pump, and designed to be mobile. Every 24-h post-fertilization, the calcium-supplemented water was turned off to three consecutive aquaria, and the water replaced by moving the calcium-deficient water manifold to the three aquaria. After 24-h, calcium-supplemented water was restored, and the low-calcium manifold moved to the next group of three aquaria. The control aquaria received calcium-supplemented water throughout embryogenesis.

Egg masses were allowed to hatch to completion within individual aquaria. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was used as a proxy for embryo survival and calculated as the percentage of eggs hatched.

Statistical Analyses

Statistical comparisons for each trial were conducted using the SAS software system version 8.00 (SAS Institute Inc., Cary, North Carolina, USA). Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values, and the Shapiro-Wilkes test for normality. Hatching success data, expressed on a percentage basis, were arcsin-transformed prior to analysis of variance (ANOVA) using mixed-model procedures. Pairwise contrasts were used to identify significant differences at the 5% level among treatments at different time points.

Results and Discussion

The possibility of a calcium metering pump failure during the hatchery season is very real and a concern to commercial catfish hatcheries in the Mississippi delta where soft-water wells are commonplace. Daily hardness values from unsupplemented well water in the current study averaged 4.7 ± 1.6 mg/L. Ca-hardness levels less than 10 mg/L have been shown to have a

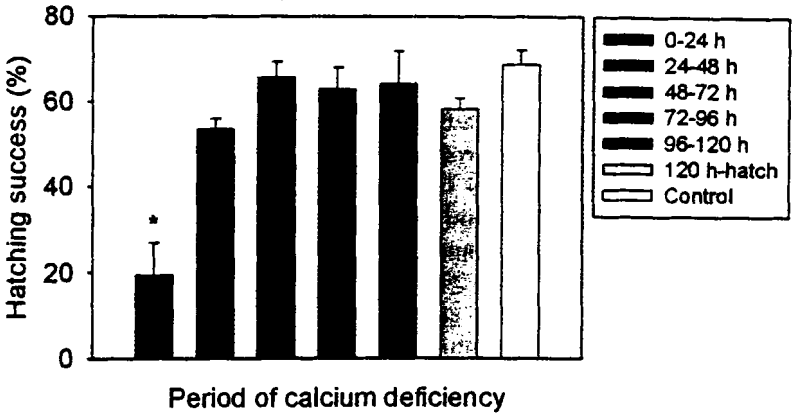

FIGURE 1. *Effect of low calcium hardness (4.7 mg/L as $CaCO_3$) in hatchery water during 24-h periods of embryogenesis on channel catfish hatching success compared to a control group receiving calcium supplementation throughout embryogenesis. The asterisk (*) indicates statistical significance ($P < 0.05$).*

significant negative effect on catfish sac-fry development and survival (Tucker and Steeby 1993), but until now, the effect of low Ca-hardness on embryo survival and hatching success has not been reported for channel catfish.

In the present study, the protocol used to spawn channel catfish and collect eggs was designed to simulate common commercial practices. By allowing the fish to spawn in the pond, the eggs were fertilized and briefly incubated in a water environment with abundant calcium (Ca-hardness, 107 mg/L; total hardness, 233; alkalinity, 227 mg/L; pH, 8.6) prior to removal to the hatchery. This methodology allowed for a practical approach to assessing the effects of Ca-hardness in hatchery water as it relates to commercial hatchery management practices in the Mississippi delta.

Hatching occurred during the sixth day post-fertilization in the present study. As a result, there were six treatment periods in which three replicate tanks each period received the low Ca-hardness water for 24 h, and six aquaria that received calcium-supplemented water throughout the study. Daily Ca-hardness averaged 94.7 ± 6.9 mg/L in the calcium-supplemented hatchery water.

Hatching success was significantly ($P < 0.05$) affected by water Ca-hardness (Fig. 1). Of the six 24-h developmental periods of embryogenesis, the first 24-h post-fertilization was determined to be the only calcium-critical period. Hatching success was 72% lower among eggs incubated in low-calcium water during the first 24-h relative to controls. Hatching success averaged 60.8 ± 2.2% among the remaining 24-h developmental periods of embryogenesis, and was statistically similar ($P > 0.05$) to the controls (68.7 ± 3.4%). The level of hatching success observed in the controls is similar to that observed in other studies conducted at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi (Small and Bates 2001; Small and Wolters 2003).

Critical stages of embryogenesis are often studied in terms of pollutants or chemical disinfectants. In this respect, critical developmental stages have been reported for developing trout embryos (Arndt et al. 2001), and similar effects have been observed with developing hybrid catfish *Ictalurus punctatus* × *I. furcatus* embryos (Nagaraj Chatakondi, Harvest Select Farms, Inverness, Mississippi, USA, personal communication). While it is generally agreed that water quality affects channel catfish embryo survival, very little is known regarding the effect on survival during specific developmental stages. Enhanced sen-

sitivity to temperature of channel catfish during early embryogenesis has been demonstrated (Small and Bates 2001). Small and Bates (2001) found that channel catfish embryos were unable to acclimate to water temperatures of 16 C or below prior to formation of the embryonic axis. Other studies suggest that routine handling of channel catfish egg masses during the first 24-h post-fertilization can reduce hatching success as much as 20% compared to unhandled controls (B.C. Small, USDA/ARS Catfish Genetics Research Unit, Stoneville, Mississippi, USA, unpublished). Together with the dependance on environmental calcium observed in the present study, these data demonstrate a critical period of development during the first 24 h of embryogenesis.

The problem of rearing channel catfish fry in soft, calcium-deficient water was identified by Tucker and Steeby (1993), and as a result of their research, calcium supplementation is now commonplace for commercial catfish hatcheries. The information presented here makes it possible to manage for improved hatching success during periods of no calcium supplementation, such as when a metering pump fails. Simple management practices might include leaving newly spawned eggs in the pond an extra day, or manually adding a calcium solution to hatching troughs designated for new spawns. This research provides further evidence of the need for calcium supplementation in hatchery water, and provides valuable information for managing an optimal hatchery environment for channel catfish.

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Literature Cited

Arndt, R. E., E. J. Wagner, and M. Douglas-Routledge. 2001. Reducing or withholding hydrogen peroxide treatment during a critical stage of rainbow trout development: effects on eyed eggs, hatch, deformities, and fungal control. North American Journal of Aquaculture 63:161–166.

Barrett, K. J., D. G. McDonald, and M. J. O'Donnell. 2001. Changes in ion content and transport during development of embryonic rainbow trout. Journal of Fish Biology 59:1323–1335.

Boyd, C. E. and C. S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Alabama Agriculture Experiment Station, Auburn University, Auburn, Alabama, USA.

Chou, M.-Y., C.-H. Yang, F.-I. Lu, H.-C. Lin, and P.-P. Hwang. 2002. Modulation of calcium balance in tilapia larvae (*Oreochromis mossambicus*) acclimated to low-calcium environments. Journal of Comparative Physiology B 172:109–114.

Flik, G., J. C. Fenwick, Z. Kolar, N. Mayer Gostan, and S. E. Wendelaar Bonga. 1986. Effects of low ambient calcium levels on whole-body Ca^{2+} flux rates and internal calcium pools in the freshwater cichlid teleost, *Oreochromis mossambicus*. Journal of Experimental Biology 120:249–264.

Makeeva, A. P. and N. G. Emel'yanova. 1993. Early development of the channel catfish, *Ictalurus punctatus*. Journal of Ichthyology 33:87–103.

McCormick, S. D., S. Hasegawa, and T. Hirano. 1992. Calcium uptake in the skin of a freshwater teleost. Proceedings of the National Academy of Sciences USA 89:3635–3636.

NASS (National Agricultural Statistics Service). 2002. Catfish production, 07.29.02. USDA-NASS, Washington, D.C., USA.

Patrick, M. L., C. M. Wood, and W. S. Marshall. 1997. Calcium regulation in the freshwater-adapted mummichog. Journal of Fish Biology 51:135–145.

Perry, S. F. and C. M. Wood. 1985. Kinetics of brachial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. Journal of Experimental Biology 116:411–433.

Small, B. C. and T. D. Bates. 2001. Effect of low-temperature incubation of channel catfish *Ictalurus punctatus* eggs on development, survival and growth. Journal of the World Aquaculture Society 32:49–54.

Small, B. C. and W. R. Wolters. 2003. Hydrogen peroxide treatment during egg incubation improves channel catfish hatching success. North American Journal of Aquaculture 65:314–317.

Tucker, C. S. and J. A. Steeby. 1993. A practical

calcium hardness criterion for channel catfsih hatchery water supplies. Journal of the World Aquaculture Society 24:396–401.

Weirich, C. R. and T. R. Tiersch. 1997. Effects of environmental sodium chloride on percent hatch, yolk utilization, and survival of channel catfish fry. Journal of the World Aquaculture Society 28: 289–296.