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Effect of Low-Temperature Incubation of Channel Catfish *Ictalurus punctatus* Eggs on Development, Survival, and Growth

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Abstract.—To determine whether the embryonic period of channel catfish *Ictalurus punctatus* could be extended at low temperatures, fertilized channel catfish eggs were incubated at five constant water temperatures: 4, 11, 16, 21, and 26 C. Low-temperature incubation of catfish eggs extended the embryonic period at 16 (244%) and 21 C (56%) when compared to the control hatchery incubation temperature of 26 C. All eggs incubated at 4 and 11 C died within 24–48 h. Developmental stage had a significant ($P < 0.05$) effect on percent hatch at 16, 21, and 26 C. Eggs held at 16 C prior to embryonic axis formation died within 48 h. Larvae from eggs hatched at 16 C were incompletely developed and died upon acclimation to 26 C for growth tests. Growth of fry reared at 26 C, following egg incubation at 21 C, paralleled that of fry from eggs incubated at 26 C. The underdevelopment of fry at 16 C combined with the significant effect of egg stage on survival at this temperature suggests that 16 C is below the lower thermal tolerance limit for normal development in this species. The period prior to the formation of the embryonic axis may be considered a vulnerable stage in channel catfish development. Increasing the embryonic period through low temperature incubation would increase the duration of juvenile availability for researchers and commercial operations.

Temperature is an important environmental factor affecting fish embryo development and the survival and growth of fish larvae. Many studies have examined the effect of temperature on the early development and survival of cultured and wild fishes (Pauly and Pullin 1988; Pepin 1991; Kucharczyk et al. 1997; Pepin et al. 1997). Increased temperature within an optimal range results in faster development and shorter time to hatch. Incubation temperatures outside a species optimal range have been demonstrated to have severe detrimental effects on hatchability and survival (Kucharczyk et al. 1997; Kujawa et al. 1997; Buckley et al. 2000).

Channel catfish *Ictalurus punctatus* is an important freshwater aquaculture species. These fish typically spawn in the spring when water temperatures are between 21 and 29 C. At temperatures of 27–28 C, the embryonic period lasts an average of 5 d (Makeeva and Emel'yanova 1993). The embryonic period may be increased by incubating eggs at lower temperatures, and thus decreasing the metabolic rate of the developing embryos. The short incubation period of channel catfish embryos might suggest that a delay in development would be limited; however, the short embryonic period of Atlantic cod *Gadus morhua* (11 d at 8 C) has been successfully extended an additional 27 d by lowering the incubation temperature 7 C (Buckley et al. 2000).

The present study examined the effects of low water temperature on the progression of early life stage development in channel catfish. Survival, morphological development, and time to hatch were assessed at five temperatures from 4 to 26 C. Growth rates of viable larvae were compared for 6 wk following post-hatch acclimation to 26 C.

Materials and Methods

Spawning and Egg Collection

Channel catfish brood fish were stocked into 0.1-acre ponds in Stoneville, Mississippi, USA at a stocking density of 20 females and 10 males. Eight spawning containers were placed into each pond, and fish were allowed to spawn naturally. Spawning containers were checked every 2 d for egg masses. Egg masses used in this study were collected during the first 2 wk of June. 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 prior to temperature acclimation.

Incubation System

The incubation system was composed of four Living Stream raceways (LS-510, Frigid Units Inc., Toledo, Ohio, USA) equipped with separate heater/chiller units to maintain constant water temperature. Each raceway was equipped with four airstones and four suspended baskets for containment of individual egg masses and hatching larvae. Two aerated 10-L aquaria were placed in a refrigeration unit for incubation of eggs at 4 C. Each raceway and aquarium was a closed system. Water circulation was maintained through vigorous aeration and recirculation through the heater/chiller units.

Egg Acclimation and Incubation

Twenty eggs from each of four spawns were collected and photographed under a microscope to determine egg stage. The number of eggs per gram was determined for each spawn. Individual egg masses were then subdivided into batches of similar mass and acclimated to the respective water temperature, such that each spawning group was represented among all temperatures. Acclimation was accomplished by placing the egg masses in the respective systems, initially at 26 C, and then allowing the chillers or refrigeration unit to lower the water temperature at a rate of approximately 2 C/h. Formalin baths (1.5 mL/L for 15 min) to prevent fungal infection were administered every 2 d beginning 1 d after temperature acclimation and continuing until embryonic eye pigmentation became apparent. Eggs (N = 10) were collected every 2 d for developmental staging.

Fry Growth

Egg masses were allowed to hatch to completion within the individual baskets. When hatching was complete, the larvae were siphoned into a graduated cylinder and the volume of larvae recorded. The larvae were then acclimated to 26 C at a rate of approximately 2 C/h and placed in 38-L aquaria to determine the effect of egg incubation temperature on fry growth. At the onset of swim-up, 20 fry from each replicate group were collected for length and weight determination, and the population in the aquarium was reduced to 100 fry. Feed was offered 4 times daily to apparent satiation. Measurements of weight and length (N = 10) were conducted again at 3 and 6 wk following initial swim-up. This schedule was consistent for each replication of the 21 and 26 C treatments.

Statistical Analysis

Percent hatch, weight and length were subjected to analysis of variance (ANOVA) mixed-model procedures 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. The data were found to be both homogeneous and normally distributed. Time to hatch at the different temperatures was fitted to an exponential curve using SigmaPlot software version 4.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Embryonic development was greatly influenced by water temperature. For descriptive purposes, developmental stages were defined according to Markle and Frost (1985). At incubation temperatures of 4 and 11 C, all eggs died within 24–48 h of acclimation. Survival at 16 C was dependent on developmental stage at the time of acclimation. Of the four spawns, two contained eggs acclimated prior to formation of the embryonic axis (Stage I), and two contained eggs in which the embryonic axis was apparent (Stage II). All of the Stage I

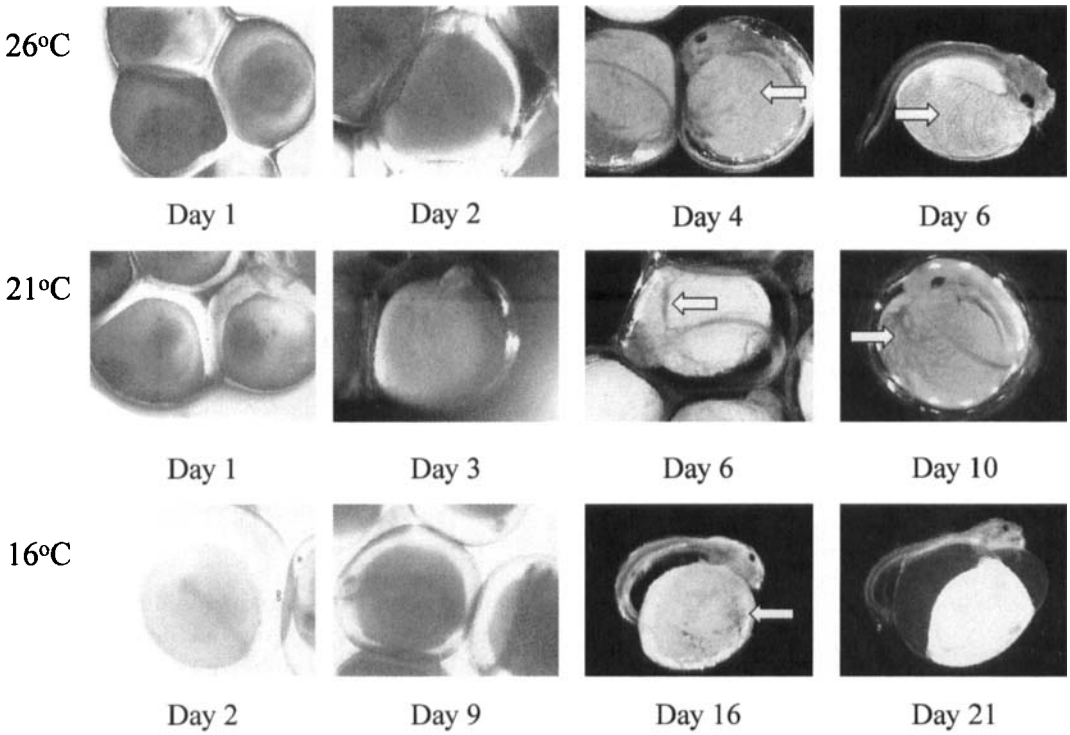

FIGURE 1. *Development of channel catfish embryos at three incubation temperatures: 26, 21, and 16 C. Embryos shown at 16 C were acclimated 2 d post-fertilization to increase survival. Arrows emphasize vascular system development. Many branching capillaries can be seen covering the yolk-sac of embryos reared at 26 and 21 C. Poor vascular development, differential yolk absorption, and emaciation were apparent at 16 C.*

egg masses died within a period of 24–48 h after acclimation to 16 C. In contrast, eggs in developmental Stage II survived to hatch at 16 C.

Embryos incubated at 21 and 26 C demonstrated normal developmental characteristics, while abnormal vascular development, differential yolk utilization and emaciated embryos were observed in the two surviving 16 C egg masses (Fig. 1). Embryos incubated at 26 and 21 C had well developed vascular systems by days four and six, respectively, made apparent by the many branching capillaries covering the yolk sac and the observation of blood pumping through the vitelline vein. It was not until day 16 that blood became visible in embryos incubated at 16 C. Even then, these embryos never developed a well defined provisional vascular system in the yolk. By day 21, neither a provisional vascular system nor any sign of hemoglobin was apparent. Furthermore, the yolk sacs of embryos incubated at 16 C appeared abnormal, and contained a large amount of clear fluid.

The effect of temperature on the duration of embryonic development was significant ($P = 0.0012$); the embryonic period increased with decreasing temperature. Egg stage at the time of temperature acclimation had no effect on time to hatch ($P = 0.35$). The time from fertilization to completion of hatch averaged 6.25 d ($N = 4$) at an incubation temperature of 26 C, 9.75 d ($N = 4$) at 21 C, and 21.5 d ($N = 2$) at 16 C (Figs. 1, 2), and was fit to an exponential curve (Fig. 2). The duration of hatching was also prolonged at lower incubation temperatures. At 26 C, hatching was complete within sev-

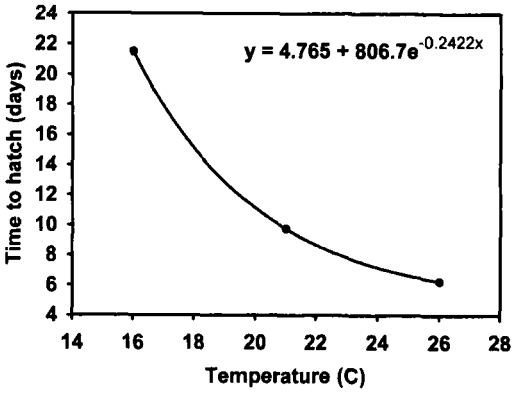

FIGURE 2. *Regression curve demonstrating effect of three incubation temperatures (16, 21, and 26 C) on time to hatch channel catfish eggs (N ≥ 2). The effect of temperature was significant (P = 0.0012). At 26 and 21 C, the effect of egg stage at the time of temperature acclimation was not significant (P = 0.35). Stage I eggs (Markle and Frost 1995) did not survive acclimation to 16 C.*

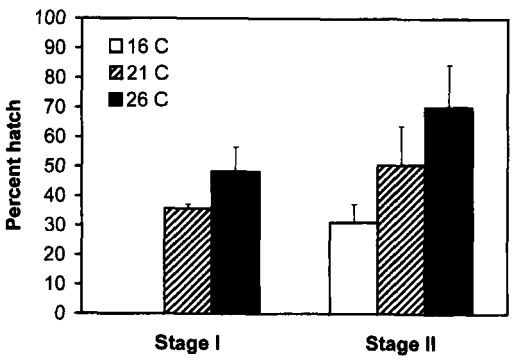

FIGURE 3. *Effect of developmental stage, as defined by Markle and Frost (1995), and incubation temperature on percent hatch of channel catfish eggs. Values are means ± SEM (N ≥ 2). There were significant effects of temperature (P = 0.018) and stage (P = 0.041).*

eral hours. At 21 C, hatching continued beyond 24 h, and at 16 C, the duration of hatching was greater than 48 h.

Developmental stage at the time of hatchery acclimation had a significant effect on percent hatch at 16, 21, and 26 C (Fig. 3). Eggs removed from spawning containers while in developmental Stage I had lower percent hatch than did eggs collected in Stage II (P = 0.041). Incubation temperature also affected percent hatch (P = 0.018), decreasing percent hatch at lower temperatures.

In order to determine the effect of egg incubation temperature on growth, larvae hatched at 16 and 21 C were acclimated to 26 C for comparison to the 26 C treatment. Larvae from eggs hatched at 16 C died during acclimation to 26 C, resulting in the growth comparison of only the 21 and 26 C treatments. The rate of gain for weight and length was similar in both treatments, being parallel over time with no differences in weight or length relative to days post hatch (Figs. 4, 5).

Discussion

In an effort to determine whether the embryonic period of channel catfish could be extended by low incubation temperatures, this study found not only significant effects of temperature on embryo development, but also significant effects of egg stage. Embryos incubated at 21 and 16 C took 56 and 244% longer, respectively, to complete hatching than those at 26 C. However, development of embryos hatched at 16 C was anomalous and larvae were not viable. Many studies have documented critical temperature extremes at which embryo development is altered or ceases altogether (Kawahara et al. 1997; Kucharczyk et al. 1997; Kujawa et al. 1997). The underdevelopment of fry at 16 C combined with the significant effect of egg stage on survival at this temperature suggests that 16 C is below the lower thermal tolerance limit for normal development of channel catfish embryos.

Decreasing temperature not only extended the embryonic period, but also had a negative effect on percent hatch. On average, eggs incubated at 21 C yielded 27% less viable offspring compared to those hatched at 26 C. While this decrease in percent hatch is not easily explained, it might be related to the importance of egg stage at the time of temperature acclimation. Our data suggest that development prior to Stage II is temperature critical. None of the

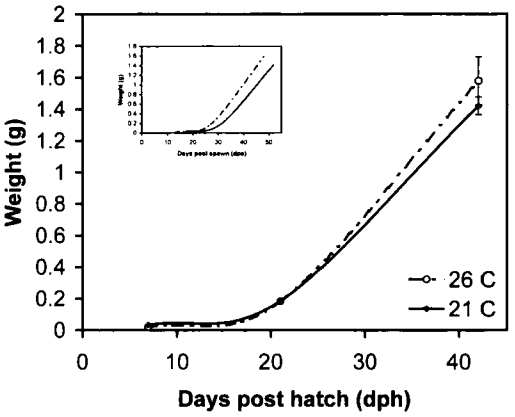

FIGURE 4. *Effect of two egg incubation temperatures (21 and 26 C) on body weight of channel catfish fry reared at 26 C relative to the number of days post hatch. Inset shows parallel growth rates relative to days post spawn. Values are means ± SEM (N = 4). Treatment means were not significantly different (P > 0.05).*

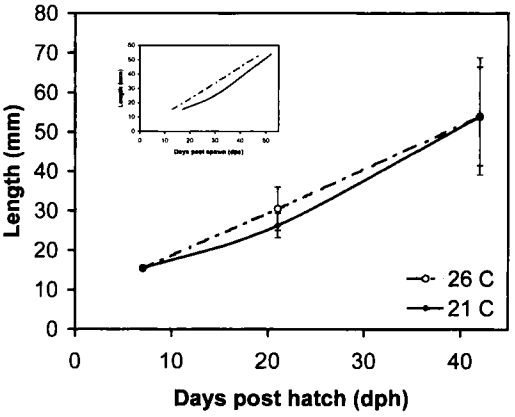

FIGURE 5. *Effect of two egg incubation temperatures (21 and 26 C) on total length of channel catfish fry reared at 26 C relative to the number of days post hatch. Inset shows parallel growth rates relative to days post spawn. Values are means ± SEM (N = 4). Treatment means were not significantly different (P > 0.05).*

Stage I eggs acclimated to 16 C survived, while Stage II eggs hatched at 16 C. Egg stage not only had an effect on survival at 16 C, but also at 21 and 26 C, suggesting Stage I eggs may be more sensitive to handling stress. Latif et al. (1999) observed egg mortality in walleye *Stizostedion vitreum* during various developmental stages and concluded that the highest rate of mortality occurred during the transformation of germinal layers into various organs of the embryo (gastrulation-early organogenesis). Based on observations of salmonid embryos, Hayes (1949) suggested that growth and differentiation during organogenesis become uncoordinated by a substantial deviation in temperature beyond optimum. Our own observations indicate greater survival at hatch if the eggs were collected after formation of the embryonic axis.

Considering that channel catfish typically spawn when water temperature is between 21 and 29 C, it was not surprising that embryo development was hindered below 21 C. Determining the degree of developmental hindrance was important in answering the question of whether the embryonic period could be successfully extended to yield viable offspring. Extending the incubation period could benefit the catfish aquaculture industry by allowing researchers and commercial growers access to juveniles over a longer time period.

In this study, we demonstrated that the duration of viable egg and larval availability could be extended by as much as 56% at 21 C. However, this increase in duration does have a cost; decreasing incubation temperature resulted in decreased percent hatch. The observation that the period prior to the formation of the embryonic axis is a temperature critical developmental stage in channel catfish, together with the observation that egg masses collected from the ponds prior to this stage yielded lower percent hatch, lends itself to further investigation. Managers aware of water temperatures might alter the intervals for collecting eggs to ensure that most eggs have reached Stage II. Such practices during periods of cool temperatures could increase survival and reduce labor costs.

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