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[Communication]

Application of a Temperature-Dependent Mitotic Interval (τ_o) for Induction of Diploid Meiotic Gynogenetic Paddlefish

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Abstract.—We tested the application of mitotic interval (tau [τ_o]) unit in comparison with absolute time to help standardize preshock timing for a consistent production of diploid meiotic gynogenetic paddlefish *Polyodon spathula*. The diploid gynogenetic larvae were produced by applying heat shock (35°C; 2 min) at different times after activation of paddlefish eggs with irradiated sperm of shovelnose sturgeon *Scaphirhynchus platorynchus* at two different preshock temperatures (15°C and 20°C). When the timing of heat shock (minutes after activation) was expressed in absolute time, the yield of gynogenetic diploid larvae was distinctly different between the two preshock incubation temperatures. At 20°C, the highest yield was observed when the timing of the heat shock was 12–13 min after activation, whereas at 15°C the highest yield was observed when the timing of the heat shock was 17–22 min after activation. However, when the timing of heat shock was expressed in terms of τ_o, the yield of gynogenetic diploid larvae for the two preshock incubation temperatures coincided completely at each time point tested. The highest yield (about 30%) of diploid gynogenetic larvae was obtained when the timing of heat shock was between $0.22\tau_o$ and $0.26\tau_o$. Reporting of heat shock timing in terms of τ_o provides the opportunity to standardize the preshock time interval for any preshock temperature. This could help to identify the optimal timing of heat shock for a specific experiment and allow comparison of data among different experiments in which different preshock temperatures were used.

Meiotic gynogenesis is embryological development without a paternal contribution and involves the retention of the second meiotic polar body as a way of restoring diploidy (Shelton 1989). Gynogenesis is achieved through the use of genetically inactivated (usually irradiated) sperm, and diploidy in the activated egg is then restored by a cold or heat shock or by hydrostatic pressure (Purdom 1983; Nagy 1987). Gynogenesis in paddlefish *Polyodon spathula* has been achieved through the use of ultraviolet (UV) treated spermatozoa from shovelnose sturgeon *Scaphirhynchus platorynchus* to activate paddlefish eggs, which are then diploidized by heat shock (35°C) to retain the second polar body (Mims et al. 1997). If preshock incubation water temperature is not the same each time, the absolute time of heat shock must be adjusted by use of a temperature-dependent index of mitotic interval, or tau (τ_o) (Dettlaff and Dettlaff 1961).

A τ_o unit is the duration (min) of one mitotic cycle during synchronous embryonic cleavage. To calculate the duration of a mitotic cycle in acipenserids, the absolute time elapsed between the appearance on the egg surface of the first and second cleavage and the time elapsed between the second and third cleavage are averaged (Dettlaff 1991). Shelton et al. (1997) described a temperature-dependent relationship of τ_o for paddlefish to help standardize chromosome manipulation in eggs and determined the value of τ_o for different water temperatures. Mims et al. (1997) found that the highest yields of gynogenetic larvae occurred when the activated eggs, preincubated at 16°C or 18°C, were heat shocked at $0.26\tau_o$ (18.7 or 16.5

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min after egg activation, respectively) for 2 min and then were returned to a temperature of 18°C. Theoretically, when shock timing (τ_n, min) at a specific temperature is divided by τ_o (absolute time of one mitotic cycle), the dimensionless quotient (τ_n/τ_o) should be a constant at any preshock temperature.

Previous research by Mims et al. (1997) demonstrated the utility of τ_o based at preshock temperatures of 16°C or 18°C to improve treatment standardization for consistent induction of paddlefish gynogenesis. However, many hatcheries usually operate at a wider temperature range. The objective of this study was to further assess the utility of τ_o as a method of adjusting absolute shock time at two more divergent preshock incubation temperatures, 15°C and 20°C, which are considered to be the lowest and highest temperature limits for normal incubation of paddlefish embryos (Mims 2001). In this study, we also applied heat shocks in a larger time interval after activation ($0.18–0.38\tau_o$) than was used in a previous study (Mims et al. 1997).

Methods

Mature male shovelnose sturgeon and female paddlefish were captured below Uniontown Dam on the Ohio River. Broodfish were transported to the Aquaculture Research Center, Kentucky State University, Frankfort, and were held in separate 2.5-m-diameter tanks. Each tank was supplied with dechlorinated water at a flow rate of 12 L/min and a water temperature of 18 ± 0.5°C. Two female paddlefish were injected intraperitoneally with luteinizing hormone releasing hormone analog (LHRH-A: des-gly10[D-ala6]-LHRH) at a dose of 100 μg/kg in a series of two injections (10 and 90 g/kg) separated by a 12-h interval. Six shovelnose sturgeon males were given a single injection of LHRH-A at a dose of 50 μg/kg after the second injection was administered to the female paddlefish. Female paddlefish ovulated within 16–18 h; shovelnose sturgeon began to spermiate within 12 h.

Milt was collected in 3-mL syringes and immediately placed on wet ice. Individual milt samples were examined under a light microscope, and the best three samples with near 100% motility were pooled. Shovelnose sturgeon spermatozoa were irradiated with UV light to deactivate the genome without affecting motility, as described by Mims and Shelton (1997). Irradiated milt was placed on wet ice in a dark, insulated container until eggs were ready to be activated. Motility was re-checked prior to egg activation.

Eggs from two female paddlefish were collected, pooled, and divided by weight into three batches of 40,000 eggs/batch. One batch of eggs was fertilized with fresh paddlefish sperm and was used as a control for evaluation of egg quality (control group C1). The other two batches of eggs were activated with irradiated shovelnose sturgeon sperm to induce gynogenetic development. A water temperature of either 15°C or 20°C was used for activation of these two batches and was maintained until application of heat shock. After 1 min, each batch of eggs was coated with a clay suspension for 10 min to eliminate adhesiveness. Eggs from each batch were then volumetrically subdivided into seven experimental groups (5,500 eggs/group). Eggs from one group were not subjected to heat shock (control group C2). Eggs from the other six groups were subjected to heat shock at different postactivation times expressed in terms of τ_o: 0.18, 0.22, 0.26, 0.30, 0.34, or 0.38 × τ_o. The τ_o is 80 min at 15°C and 52 min at 20°C (Shelton et al. 1997). Each aliquot was poured into a screen-bottom floating tray, which was maintained at one of the two preshock incubation temperatures, 15°C and 20°C. To calculate time of heat shock in minutes, the timing expressed in τ_o (one mitotic interval) was multiplied by a fraction of the τ_o value at a given temperature. For example, at 15°C, τ_o is 80 min and $0.18\tau_o$ is equal to 14.4 min (0.18 × 80). At each of the respective heat shock treatments, the eggs in the trays were transferred and submerged for 2 min in a 300-L water bath held at 35 ± 0.3°C. After heat shock, each batch of eggs was returned to a water bath of 18°C until they were transferred into 10-L McDonald jars for further incubation.

At hatch, larvae swam up and over into hapas suspended in 100-L conical-shaped fiberglass tanks. Larvae were counted, and the percentage of viable gynogenotes was calculated for each shock timing. Analysis of variance was conducted on the data separately; timing of heat shock was expressed in both τ_o and absolute time, and preincubation temperature was the independent variables. The relationship between the percentage of gynogenetic diploid larvae and the timing of heat shock was examined by regression, and the differences between preshock incubation temperatures were examined by the homogeneity of regression test (SAS Institute, Inc. 1990).

Results

The yield of normal larvae in control group C1 was 68%. There were no viable larvae (haploid) in control group C2.

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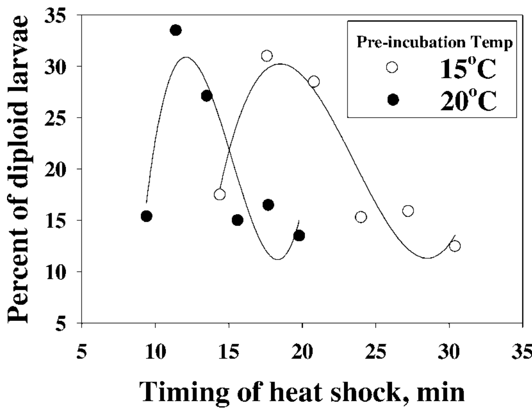

FIGURE 1.—Effect of heat shock initiation expressed in absolute time (min) on the percentage of viable, meiotic gynogenetic diploid paddlefish larvae produced from activated eggs incubated at two preshock incubation water temperatures (15°C and 20°C). Cubic equations are as follows: $Y = 0.038x^3 - 2.66x^2 + 59.72x - 402.81$ for 15°C ($r^2 = 0.88$) and $Y = 0.16x^3 - 7.42x^2 + 108.13x - 479.14$ for 20°C ($r^2 = 0.83$).

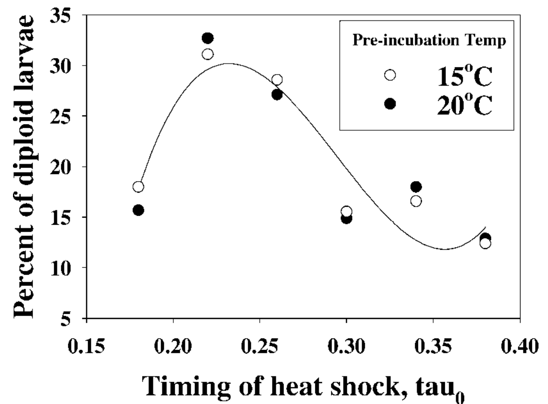

FIGURE 2.—Pooled effect of heat shock initiation expressed in mitotic interval (τ_o) on the percentage of viable, meiotic gynogenetic diploid paddlefish larvae produced from activated eggs incubated at two preshock incubation water temperatures (15°C and 20°C). The cubic equation is as follows: $Y = 19{,}083.48x^3 - 16{,}856.41x^2 + 4{,}741.60x - 400.89$ ($r^2 = 0.82$).

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The yield of normal, viable larvae in heat-shocked groups was dependent on the timing of the heat shock. When the timing of heat shock was expressed in absolute time (Figure 1), the yield of diploid larvae was affected significantly by the timing of heat shock ($P \leq 0.05$) and the preshock incubation temperature ($P \leq 0.05$). The relationship between the timing of heat shock and the yield of diploid larvae was described by cubic equations within the temperature range examined ($P \leq 0.01$). Regression lines for the two preshock incubation temperatures were not homogeneous, indicating that the data could not be combined or expressed by a single equation.

When the timing of heat shock was expressed in τ_o (Figure 2), the yield of diploid larvae was not affected significantly by the preshock incubation temperature ($P > 0.05$). The relationship between the timing of heat shock and the yield of diploid larvae was again described by cubic equations within the temperature range examined ($P \leq 0.01$). The regression lines for the two preshock incubation temperatures were homogeneous, indicating that the data could be expressed by a single equation.

Discussion

The yield of normal larvae in control group C1 was about 70%. This indicates that good-quality eggs were used in the experiment. As expected, when eggs were activated with irradiated shovelnose sturgeon sperm but were not subjected to heat shock (control group C2), no viable larvae were produced. It is known that haploid paddlefish perish at early stages of embryological development (Mims et al. 1997). The normal, viable larvae that occurred in experimental heat-shocked groups were gynogenetic diploids resulting from suppression of the second meiotic division.

The yield of diploid gynogenetic larvae was dependent on the timing of heat shock. When the timing of heat shock was expressed in absolute time (min after activation), the yield of gynogenetic diploid larvae was distinctly different between the two preshock incubation temperatures (Figure 1). At 20°C, the highest yield was observed when the timing of the heat shock was 12–13 min after activation, whereas at 15°C the highest yield was observed when the timing of heat shock was 17–22 min after activation. However, when the ''timing'' of heat shock was expressed in terms of τ_o, the yield of gynogenetic diploid larvae for the two preshock incubation temperatures completely coincided at each time point tested (Figure 2). The highest yield (about 30%) of diploid gynogenetic larvae was obtained when the timing of heat shock was between $0.22\tau_o$ and $0.26\tau_o$. This is in agreement with the preliminary results by Mims et al. (1997), which indicated that the highest yield was at $0.26\tau_o$.

Optimum induction of chromosomal manipulation at the metaphase–anaphase meiotic stages of nuclear division has the greatest importance to

successful gynogenetic production. Preshock incubation temperature changes the rate of development and therefore changes the optimum absolute time for shocking. In the literature, sex manipulation studies on fish often reported the timing of heat shock in absolute time after activation (Ihssen et al. 1990). This makes it difficult to determine the timing of heat shock when different preshock incubation temperatures are used.

The reporting of heat shock timing in terms of τ_o provides the opportunity to standardize the preshock time interval at any preshock temperature for paddlefish as well as for other fish species. This could help to identify the optimal timing of heat shock for a specific experiment and also allows the comparison of data from different experiments in which different preshock temperatures are used.

Acknowledgments

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