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Induced meiotic gynogenesis in shovelnose sturgeon

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Gynogenesis was induced in three shovelnose sturgeon (*Scaphirhynchus platorynchus*) by heat shock after egg activation with UV-treated paddlefish (*Polyodon spathula*) milt. Ultraviolet dosage (J m^{-2}) for the pooled milt samples was calculated using the following linear regression equation: Dosage = $2405.27 - 352.80X + 19.78X^2$ (X = percent transmittance of milt). Activated eggs were incubated at 18 °C until shocking at 35 °C. Shock duration was applied at $0.05\tau_0$ intervals from 0.15 to 0.40 τ_0 (8.25 to 22.00 min post-fertilization; τ_0 at I8 °C = 55 min). The highest yield of gynogenotes (16%) was observed at 0.25 τ_0 for female 3, 10 % at 0.30 τ_0 for female 2 and 12% at 0.35 τ_0 for female 1. The percentage of viable gynogenotes responded quadratically to the tau index (τ_s/τ_0) when shock treatments were applied. The higher yields of viable diploid sturgeon gynogenotes were achieved when eggs were heat shocked at embryological ages ranging from 0.25 to 0.35 τ_0 (approximately 14 to 19 min post-activation at 18 °C). No viable hybrids were produced in the control fertilization of sturgeon eggs with intact paddlefish sperm which verified the gynogenetic origin of the offspring produced.

KEYWORDS: Induced gynogenesis, Paddlefish (*Polyodon spathula*) milt, Shovelnose sturgeon (*Scaphirhynchus platorynchus*)

INTRODUCTION

Female chondrosteans, sturgeons (Acipenseridae) and paddlefish (Polyodontidae) are highly valued for their roe as caviar. Of the 26 sturgeon species and two paddlefish species found in the world, only several Caspian Sea sturgeon species are the main source of sturgeon caviar for world production (Sternin and Dore, 1993). Demand for captive breeding and aquaculture of sturgeons to produce caviar has increased as natural stocks are depleted or closed to commercial harvest, However, it often takes 10 to 20 y before a female sturgeon is mature and capable of producing roe for caviar harvest. Further, because the normal distribution of the sex is 1:1 (female to male), half the fish (males) in the culture population would have little economic value. Production of all-female sturgeon would be more economical than producing both sexes (USDA/CSRS, 1988).

Techniques of induced gynogenesis (Mims *et al.*, 1997) and sex reversal of paddlefish (Mims *et al.*, 1995) have been developed to ultimately establish a

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breeding program using neomale (sex-reversed gynogenotes) to produce all-female offspring. Mims *et al.* (1997) have reported that the gynogenetic progenies of paddlefish are all-female. These data indicated the occurrence of female homogamety (and correspondingly male heterogamety) in this species. On this basis, it may be suggested that the sturgeons, also belonging to chondrosteans, have the same mechanism of sex determination and similar techniques could be potentially applied to them for caviar production.

The shovelnose sturgeon *Scaphirhynchus platorynchus* is indigenous to the Mississippi River drainage including 21 states of the United States (Wallus, 1990). It is a small species (<3 kg) that can sexually mature in 5 to 6 y, i.e. much more quickly than most other sturgeons. Culture of all-female shovelnose sturgeon by direct induction of gynogenesis would provide greater economic returns than mixed-sex populations as well as serve as a model for other commercially important sturgeons in monosex culture. The objective of this study was to develop a gynogenetic technique that would provide direct induction of all-female shovelnose sturgeon.

MATERIALS AND METHODS

Broodstock and spawning induction

All experiments were conducted at the Aquaculture Research Center (ARC), Kentucky State University, Frankfort, Kentucky, U.S.A, in 1996 and 1997. Shovelnose sturgeon from 1 to 2.5 kg were captured during the spring migration below Smithland and Uniontown Dams on the Ohio River. Male brood paddlefish from 7 to 12 kg were also captured during spring migration below Uniontown Dam on the Ohio River and Cumberland Lake, Kentucky U.S.A. Broodfish were transported to and held in 400 m^2 holding ponds at ARC. In both years, three female and 10 male sturgeon (males used only for sturgeon egg quality control) were separated by sex and kept in two circular metal tanks (3000 l) with water flow rate of 12 l/min 9.0 mg O_2/l and controlled water temperature of 17 to 19 °C for 3 days. Three male paddlefish were selected and held separately in 3000 l circular metal tanks with water flow rate of 12 l min^{-1}, 9.0 mg O_2/l, and controlled water temperature of 17 to 19 °C for 3 days. Broodfish were injected intraperitoneally with LHRH analogue of des Gly10(D-Ala6) LHRH ethylamide (Graham *et al.*, 1986). Female sturgeon received a total dosage of 100 μg/kg body weight (BW) administered in a priming injection (10 μg/kg BW) and a resolving injection (90 μg kg^{-1} BW) 12 h apart. Male paddlefish and sturgeon received a single dose of 50 μg kg^{-1} BW when the females were given the priming injection.

UV-irradiation of paddlefish spermatozoa

Genetically inactivated UV-irradiated spermatozoa were used for insemination of shovelnose sturgeon eggs in gynogenetic trials. The use of irradiated heterologous spermatozoa insured that the viable offspring were of gynogenetic origin, because the hybrid (Shovelnose sturgeon X Paddlefish) was known to be completely inviable (Mims *et al.*, 1997).

Paddlefish milt samples varied greatly in the number of spermatozoa (0.6 to $1.5 \times$ ml^{-1}) and were much more dilute than milt of teleosts. Our preliminary experiments

demonstrated that because of this naturally dilute milt, it was impossible to use only one certain UV-dose for genetic inactivation of paddlefish sperm (as usually applied to sperm for inducing gynogenesis in teleosts). Instead, the dosage of irradiation had to be adjusted according to the density of spermatozoa (correlated with light transmittance) in any given milt sample that was used for each gynogenetic trial. By using this special technique (Mims and Shelton, unpublished) as developed for sperm of shovelnose sturgeon (Mims *et al.*, 1997), the dependence of the effective dose of UV-irradiation on the sperm concentration was determined.

In brief, milt was collected in 10 ml syringes and immediately placed on wet ice. Individual milt samples were examined under a light microscope at 200X in dark field and only those samples determined to have 90–100% spermatozoal motility were combined (Mims, 1991; Linhart *et al.* 1995). A portion of the pooled sample was placed in a polystyrene disposable cuvette and measured for percent transmittance with a Milton Roy Digital Spectronic 401 spectrophotometer (Rochester, NY U.S.A.). Since percent transmittance correlates with density of spermatozoal cells, the transmittance was used as an independent variable on which to base the dosage of UV. Effective dose of ultraviolet-irradiation was determined for 12 different values of milt transmittance (Fig. 1). Fisher UV crosslinker (Cincinnati, Ohio U.S.A.) was used as the source of ultraviolet light. Ultraviolet dosage (J m^{-2}) for the pooled sample was calculated using the following linear regression equation: Dosage (J m^{-2}) = 2405.27 − 352.80X + 19.78X^2 (X = percent transmittance). For the following

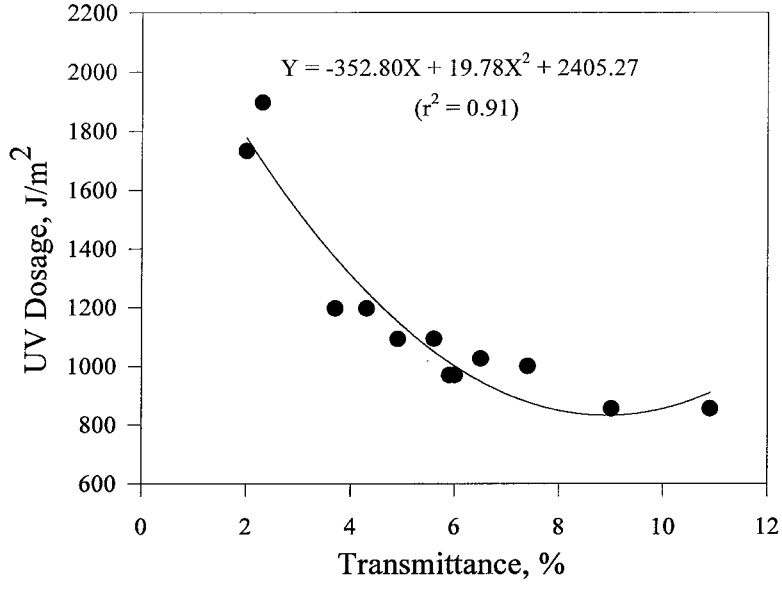

FIG. 1. Ultraviolet dosage for deactivating paddlefish sperm genome according to light transmittance (%) of milt sample. Transmittance correlates to sperm density and was determined with a spectrophotometer.

heat shock experiments, the light transmittance (%) of each milt sample was determined; and, the calculated dose was used on 2 ml milt aliquotes stored in 5 cm glass petri dishes. Irradiated milt was placed in the dark on wet ice in an insulated container until eggs were to be activated. Motility of spermatozoa was confirmed microscopically before and after UV treatment.

Activation and incubation of eggs

Sturgeon eggs (1,500 to 2,000 eggs per trial) were activated with UV-irradiated paddlefish milt and maintained at 18 (0.3) °C. Groups of eggs were held in screen-bottom wood-frame boxes submerged into a 300 l water bath until each batch was heat shocked at 35 (0.2) °C for a 2 min duration. Shock intensity and duration were selected based on optimized protocol for paddlefish (Mims *et al*., 1997). Time of heat shock ranged from 8.25 to 22.00 min in 2.75 min intervals or in mitotic intervals of 0.15 to 0.40τ_0 (τ_s/τ_0; where τ_s = time of shock in min and τ_0 @ 18 °C was 55 min; Detlaff and Detlaff, 1961; Shelton *et al*., 1997) in 0.05τ_0 increments, respectively. Three gynogenetic trials were conducted, each using gametes from a different female sturgeon (designated as female 1, 2 and 3) and a different male paddlefish. Each trial had two controls: C_1 – Sturgeon X Sturgeon (intact sperm and no shock) to verify the quality of the eggs and C_2 – Sturgeon X Paddlefish (intact sperm and no shock) to verify no viable hybrid.

The treated eggs were incubated in 10 l McDonald jars receiving flow-through, dechlorinated tap water at 18 °C. At hatching, larvae swam out of the hatching jars into hapas (net enclosures) suspended in I00 l conical-shape fiberglass tanks. Larvae were counted and percentage of viable gynogenotes calculated based on control hatching. Percentage of hatch were analyzed by general linear model (GLM) for a completely randomized block design. The relationship between percentage of viable gynogenotes and shock at mitotic intervals (τ_s/τ_0) was examined by regression (SAS, 1988).

RESULTS AND DISCUSSION

Percent hatches of C_1 were 52, 45 and 63% for females 1, 2 and 3, respectively. In all three trials for C_2, the percent hatches were 0%.

The percentages of viable gynogenotes after heat shock at different τ_s/τ_0 post-activation intervals are individually indicated in Fig. 2. The highest yield of gynogenotes (16 %) was observed at 0.25 τ_0 for female 3, 10% at 0.30 τ_0 for female 2 and 12% at 0.35 τ_0 for female 1. It should be noted that the data on the timing of heat shock (τ_s/τ_0) obtained in different trials were very close. The highest yield of viable diploid shovelnose sturgeon gynogenotes was achieved when eggs were shocked at embryological ages ranging between 0.25 and 0.35 τ_o (approximately 14 to 19 min post-activation at 18 °C). The curve in Fig. 2 reflects the pooled data obtained in all three trials. The calculated regression equation showed that the percentage of viable gynogenotes responded quadratically to the timing of the heat shock (τ_s/τ_0) application.

Recently, Van Eenennaam *et al*. (1996) reported a technique for verification of meiotic gynogenesis and polyploidy in white sturgeon (*Acipenser transmontanus*). Though the experiment was not designed to determine the optimal parameters for

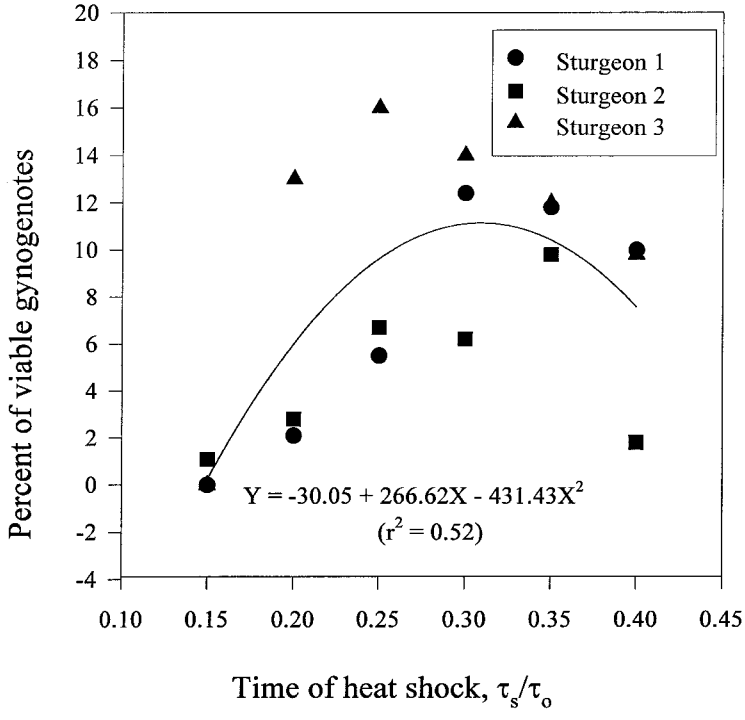

FIG. 2. The response of heat shock initiation (τ_s/τ_0) on the percentage of viable gynogenotes when pre-shock incubation water temperature was 18 (0.3) °C (where $\tau_0 = 55$ min). Data points for three replicates (0.15, 0.35 and 0.40 for females 1 and 3) are superimposed. Each data point is based on percent hatch from 1500–2000 eggs.

induction of gynogenotes in white sturgeon, highest yield of gynogenotes was obtained at 0.26 τ_0 which was similar to the results from this study. The mitotic interval for heat shocking (0.26 τ_0) white sturgeon was also most effective for paddlefish (Mims *et al.*, 1997).

The application of UV-irradiated paddlefish spermatozoa and heat shock was effective in producing viable, sturgeon gynogenotes. Without irradiation treatment of the paddlefish spermatozoa (C_2), there were no viable hybrids, which confirmed that the offspring were of gynogenetic origin. Also, Peruzzi *et al.* (1993) and Rothbard *et al.* (1997) reported the usefulness of heterologeous sperm for gynogenetic controls by demonstrating that hybridization was not viable.

Direct gynogenetic induction is usually not feasible for producing commercial quantities of monosex fish because of relatively low yield of gynogenetic offspring (comparative to usual crossing). Results from this study indicate however, that assuming female sturgeon are homogametic, direct induction could be feasible for commercial quantities of gynogenotes that potentially could provide roe for caviar. Assuming 25,000 eggs/shovelnose sturgeon female, actual numbers of gynogenotes could range from 2,500 to 4,000 fish. Also, further optimization of gynogenetic

techniques could increase the yield of viable offspring. Growth performance and gonadal development of gynogenotes will be tested in later studies, since inbreeding through gynogenesis might have some deleterious effects.

RECOMMENDATIONS

1. Shovelnose sturgeon eggs fertilized with intact paddlefish sperm should be used to prevent viable hybrids; and, therefore, will provide an appropriate control for gynogenesis.
2. Shovelnose sturgeon gynogenotes can be induced when eggs are activated with ultra-violet irradiated paddlefish spermatoza and heat shocked at 35 °C for a 2 min duration.
3. Optimal survival of shovelnose sturgeon gynogenotes can be achieved when eggs are shocked at specific embryological ages ranging between 0.25 to 0.35 τ_0 (approximately 14 to 19 min post-fertilization at 18 °C). Best calculated survival of gynogenotes (11%) was determined to be 0.30 τ_0 (16.5 min post-fertilization at 18 °C).

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