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TECHNICAL NOTES

Effectiveness of the Minimally Invasive Surgical Technique (MIST) for Removal of Ovulated Eggs from First-Time and Second-Time MIST-Spawned Paddlefish

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Abstract.—A minimally invasive surgical technique (MIST) for the removal of ovulated eggs from paddlefish *Polyodon spathula* was tested on broodstock that had been previously spawned using MIST to determine whether repeat spawning affected fecundity or hatching rate compared with broodstock than had not previously been induced to spawn. There were no significant differences in the number of eggs removed or in the hatching rate between first- and second-time MIST spawners. The oviducts from second-time MIST spawners appeared normal and had no scarring or blockage. The MIST approach is efficient, practical, and less stressful to broodstock during artificial propagation than other reported egg collection procedures.

Paddlefish *Polyodon spathula* are chondrostean fishes that have an ovary duct arrangement defined as a gymnovarian (naked-ovary) condition (Hoar 1969). The eggs are ovulated into the body cavity instead of into an ovarian cavity, and to be deposited during spawning, they must enter one of the two dorsally located ovarian funnels of the oviducts (or Mullerian ducts) to exit through the cloaca. Although most eggs are ovulated over a period of 1–2 h, the oviducts can hold only a small portion of the total collected in the body cavity (200–300 mL). The eggs from the oviducts are expelled during natural spawning, but the oviducts must be refilled from the body cavity before more eggs can be deposited.

In the artificial propagation of paddlefish, two methods have been traditionally used to remove eggs: hand stripping and caesarean section. Hand stripping is inefficient as only those eggs in the oviducts can be collected at one time. It is also labor intensive because the process must be repeated at approximate 30-min intervals over an 8–10-h period (Graham et al. 1986). Further, the long confinement and frequent handling can stress the fish. Caesarean section is a quick surgical procedure taking about 30 min to remove the eggs through an 8–10-cm abdominal incision and then suturing the incision (Conte et al. 1988). However, even when done by a professional, sutures invariably pull through the musculature and survival is typically less than 25%.

Stech et al. (1999) reported a new procedure for paddlefish that is minimally invasive, permits quick egg removal, requires little out-of-water handling time (approximately 10 min), and has resulted in nearly 100% survival. The technique is to make a small internal cut in the oviduct so that the eggs can pass directly from the body cavity through the incision and then out the urogenital opening (Figure 1). Though this minimally invasive surgical technique (MIST) has been demonstrated to be more practical than traditional procedures (i.e., hand stripping and caesarean section), this procedure has never been quantitatively evaluated with regard to effectiveness for the removal of ovulated eggs from females. Also, the repeated use of MIST on the same broodstock in subsequent spawning has not been examined.

Methods

Four MIST-treated female paddlefish that had been held since 1999 in a private, 2.5-ha pond near the Kentucky State University Aquaculture Research Center (ARC) were recaptured and transported to the ARC in April 2001. Four female fish and four male fish were caught below the McAlpine Dam on the Ohio River and also transported to the ARC. A biopsy was performed on each female fish to ensure that they were gravid (Mims and Shelton, in press). Gravid females were weighed and given a total dose of luteinizing hor-

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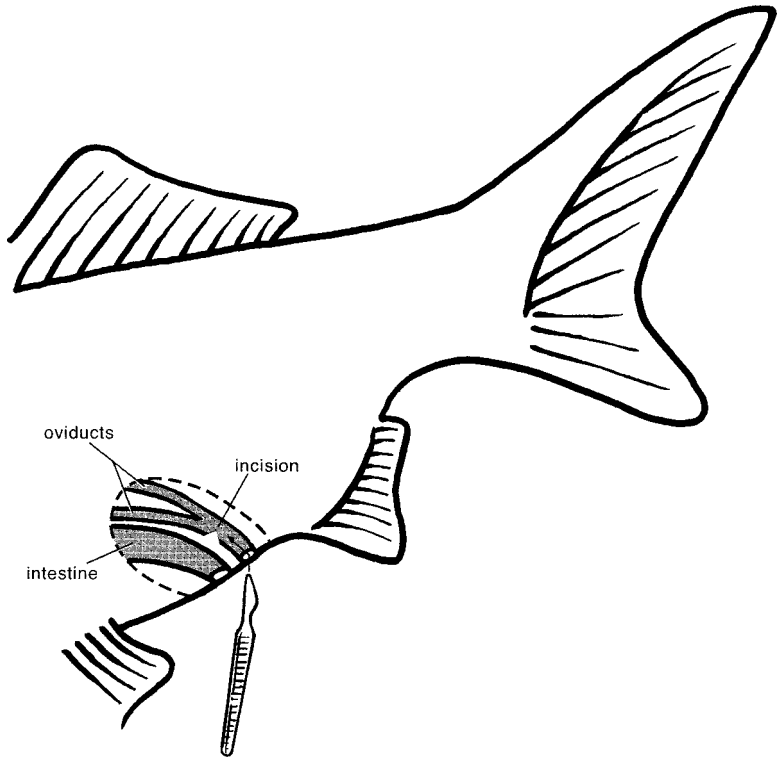

FIGURE 1.—The minimally invasive surgical technique (MIST) procedure for removal of ovulated eggs from paddlefish (Mims and Shelton, in press).

mone releasing hormone analog [$LHRH_a$; desgly^{10} (D-ala^6)-LHRH ethylamide; Sigma Chemical Co., St. Louis, Missouri] at a rate of 100 μg/kg body weight (BW) in two intraperitoneal injections (10% and 90% of the total dose) separated by 12 h. Ovulation occurred within 12–16 h after the second injection. Males were given a single intraperitoneal injection of $LHRH_a$ at a dose of 50 μg/kg BW; spermiation occurred within 12–18 h.

After the first signs of egg release, collection was delayed for about 1 h to assure that ovulation was complete. Each fish was then anesthetized with 80 mg/L of tricaine methanesulfonate (Sigma Chemical Co.). They were supported on a stretcher when out of water to facilitate handling. The MIST procedure as described by Stech et al. (1999) was performed on each female in the two treatments: (1) four fish that were previously spawned (second-timer MIST spawners) at ARC in 1999 using the MIST procedure; and (2) four fish that had not been previously spawned (first-time MIST spawners). In brief, the MIST procedure involved the insertion of a scalpel (no. 11 straight blade) about 2 cm into the urogenitial opening, making a 1–2-cm incision through the ventral wall of the common oviduct. The fish was then inverted and two persons applied stripping pressure to the abdominal region. Eggs passed through the incised opening of the oviduct and out of the urogenital opening. Each fish was stripped until the flow of eggs had significantly decreased or stopped, which took about 10 min. The eggs were weighed, and a subsample was weighed and retained for counting. Normally, a female would be returned to a holding tank for recovery, but in this study, each anesthetized female was sacrificed so as to measure residual ovulated eggs. The residual ovulated eggs were removed from each sacrificed female and weighed. The effectiveness of MIST (in percentage) was determined according to the number of eggs removed using this procedure to the total number of eggs removed (i.e., by MIST plus residual number) from each female. The eggs were fertilized, coated with Fuller's earth to eliminate adhesiveness, and placed in McDonald upwelling jars where they were incubated in nonrecirculating, dechlorinated tap water at 18°C (Graham et al. 1986; Mims et al. 1997). The numbers of

TABLE 1.—Weight, number, and percentage of eggs obtained using the minimally invasive surgical technique (MIST) procedure on first-time and second-time paddlefish MIST spawners.

Female (number)	Weight (kg)	Weight of eggs taken by MIST (g)	Weight of residual eggs (g)	Total weight of eggs (g)	Total number of eggs[a]	Eggs taken by MIST[b] (%)	Hatching rate (%)
First-time spawners							
1	8	750	154	904	97,632	83.0	90.1
2	12	616	462	1,078	116,424	57.1	79.4
3	11	1,370	190	1,560	168,480	87.8	85.8
4	10	794	156	950	102,600	83.6	84.4
Mean ± SE						77.9 ± 14.0	84.9 ± 4.4
Second-time spawners							
1	12.9	940	960	1,200	133,200	78.3	93.4
2	15.1	602	338	940	104,340	64.0	81.2
3	15.9	804	296	1,100	122,100	73.1	86.2
4	13.9	720	300	1,020	113,200	70.6	88.0
Mean ± SE						71.5 ± 5.9	87.2 ± 5.0

[a] Mean number of eggs = 108 ± 2.3/g and 111 ± 4.3/g for first-time and second-time spawners, respectively.
[b] Percent of eggs taken by MIST = eggs taken by MIST (stripping) ÷ (eggs taken by MIST + residual eggs after sacrifice) × 100.

hatched larvae were estimated volumetrically, and the data were analyzed using a standard *t*-test ($P \leq 0.05$).

Results and Discussion

The weight, number, and percentage of eggs obtained by the MIST procedures are presented in Table 1. There were no significant differences between the first-time MIST spawners and the second-time MIST spawners for the weight of eggs (77.9 ± 14.0% versus 71.5 ± 5.9%) or for the hatching rate (84.9 ± 4.4% versus 87.2 ± 5.0%). The oviducts of the second-time MIST spawners showed no abnormal scarring or blockage and were comparable to those of the first-time MIST spawners. The eggs were easier to strip if ovarian fluid was present than when the eggs were drier. Reduced ovarian fluid gave a lower percentage of eggs as observed in female 2 in both treatments. The injection of an artificial ovarian fluid (saline) might facilitate stripping and increase the percentage of eggs obtained, but we have not tested that procedure.

Most paddlefish broodstock are still obtained from public waters, but as the culture of paddlefish develops private fish farmers will have restricted access to these sources and there will be a greater need for maintaining broodstock in captivity. Thus, the long-term maintenance of healthy broodfish will be a priority and using the MIST procedure will reduce stress during artificial propagation.

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