



## SHORT COMMUNICATION

# Minimally invasive surgical removal of ovulated eggs from paddlefish

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Paddlefish *Polyodon spathula* is a valuable food fish for its roe, which is processed into caviar and boneless meat. Propagation of seed stock is possible using a hormonal induction procedure that was first established by Needham (1965) and later modified based on hormonal induction techniques for the white sturgeon, *Acipenser transmontanus* (Conte *et al.*, 1988). Further modifications are desirable for more efficient propagation of paddlefish on a commercial scale. Paddlefish, like other chondrosteian fishes, ovulate their eggs into the body cavity which opens through one of two ovarian funnels via Mullerian ducts. This type of ovary-duct arrangement is known as a gymnoovarian condition (Hoar, 1969). Two established methods are currently practiced to remove ova from an ovulated fish: hand-stripping and caesarean section. Hand-stripping is labour intensive and often takes three individuals 8–10 h to remove the total volume of ova (Graham *et al.*, 1986). Caesarean section is a quick surgical method taking approx 30 min to remove ova through an 8 to 10 cm abdominal incision (Conte *et al.*, 1988). However, suturing is time-consuming and stress on the incision usually results in low survival of broodstock. Because of complications with both methods of ova removal, a new procedure was developed which is minimally invasive and permits quick removal of ovulated ova from paddlefish and requires much less out-of-water handling time. This method of ova removal which involves a small incision of the oviduct and subsequent stripping of ova from the gonopore, was first performed on and described for sturgeons in Russia (Podushka, per.com.). We describe the application of this method on our practical work on paddlefish propagation in the United States of America.

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*Figure 1a.* A finger was inserted into the gonopore of a female paddlefish to temporarily stretch the opening.



*Figure 1b.* A scalpel with a no. 11 straight blade was carefully inserted into the stretched gonopore.



*Figure 1c.* A 1–2 cm incision was made through the dorsal area of the oviductal wall (a pencil was inserted in the oviduct and the pointer indicates the incision). The incision permitted the eggs to flow through the incised opening in the oviduct and out of the gonopore.



*Figure 1d.* The body cavity of a sacrificed paddlefish was absent of most eggs after the minimally invasive surgical procedure. The anterior portion of the oviduct (OD) and the post-ovulated ovary (OV) can be observed.

Mature paddlefish were caught in the Ohio river, transported to the Aquaculture Research Center of Kentucky State University, and held in 2.5 m diameter circular tanks supplied with dechlorinated water at a flow rate of  $12 \text{ l}\cdot\text{min}^{-1}$  and  $18.0 (0.3) \text{ }^\circ\text{C}$ . Female and male broodstock were injected with an analogue of releasing hormone LHRH (des-Gly 10[D-Ala<sup>6</sup>]-LHRH) as described by Shelton *et al.* (1997). Paddlefish ovulated 12–14 h after the resolving dose; males were actively spermiating after 12–18 h. Each ovulated female was anesthetized with  $80 \text{ mg}\cdot\text{l}^{-1}$  of MS-222 and placed ventral side up on a stretcher. One finger was inserted into the gonopore to temporarily stretch the opening (Fig. 1a). A scalpel (no. 11 straight blade) was carefully inserted into the opening (Fig. 1b) and, a 1–2 cm incision was made through the dorsal area of the oviductal (Mullerian duct) wall (Fig. 1c). The scalpel was withdrawn and the incision probed with one finger to ensure that the opening was not obstructed. The fish was inverted and slight pressure applied to the abdominal region by two individuals: the ova flowed through the incised opening in the oviduct and out of the gonopore. Eggs were fertilized, coated with Fuller's earth and placed in upwelling incubation units (McDonald jar) receiving non-recirculating, dechlorinated tap water held at  $18 \text{ }^\circ\text{C}$  (Graham *et al.*, 1986; Mims *et al.*, 1997). Hatched larvae were counted and recorded.

Eggs were collected from 10 ovulated fish using this minimally invasive surgical method. The time required to remove the majority of the eggs and return the female to the water was about 10 min per fish compared to a minimum of 30 min to complete the caesarean section procedure and over 8 h using the traditional hand-stripping method. Three fish were sacrificed after this surgical method to examine the body cavity (Fig. 1d). Only small quantities of eggs (less than 5% of total egg volume) remained in these fish. The other seven fish were stocked in ponds and resumed normal swimming within minutes with no apparent ill effects. Percentage hatch ranged between 74 and 95% which was similar to that from fertilized eggs removed by hand-stripping or caesarean section. After one month broodstock that underwent this surgical method had no secondary infection whereas fish that were hand-stripped had more extensive surface abrasions and those that underwent the more invasive caesarean section method frequently suffered infections and died.

There are a number of advantages to this minor surgical procedure for egg removal from paddlefish: (1) egg removal is rapid so that broodstock are out of the water only 5–10 min; (2) minimally invasive surgery does not require time-consuming suturing; (3) if ovulation is not complete, broodstock can be returned to the holding tank and re-examined later when ovulation is complete; (4) broodstock appear to be no more stressed than with a single hand-stripping and no mortalities have resulted; and (5) inflammation or infections were not observed compared to complications observed on fish that were used in the other traditional methods.

Our experience with this method has indicated that collection of ovulated eggs from paddlefish is reliable. Furthermore the method will permit use of broodstock for repetitive propagation.

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