CRYOPRESERVATION PROTOCOL DEVELOPMENT IN DELTA SMELT (*Hypomesus transpacificus*)

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ABSTRACT

Cryopreservation of Delta Smelt sperm was evaluated using four extenders and three cryoprotectants to identify conditions that maintain sperm quality and offspring performance. FREEZESOL and HBSS supported the highest sperm motility, and methanol preserved post-thaw sperm activity. Fertilization success was lower with cryopreserved sperm than with fresh sperm. However, hatching rate and larval survival did not differ between treatments, indicating that cryopreservation did not impair early-life performance.

BACKGROUND

Cryopreservation of sperm is a critical tool for conserving endangered fishes, yet most protocols are evaluated only up to fertilization. Delta Smelt, a federally listed endangered species, requires a reliable cryopreservation method that maintains not only sperm function but also early-life performance of offspring.

OBJECTIVES

- Develop a cryopreservation protocol for Delta Smelt
- Compare extenders and cryoprotectants for sperm quality
- Evaluate fertilization success and larval survival

METHODS

- Milt collected from hatchery males at 4 °C
- Extenders: FREEZESOL, HBSS, Ringer, HEPES
- Cryoprotectants: methanol, DMSO, glycerol
- Sperm motility measured using OpenCASA
- Fertilization and larval survival evaluated at 40 dph

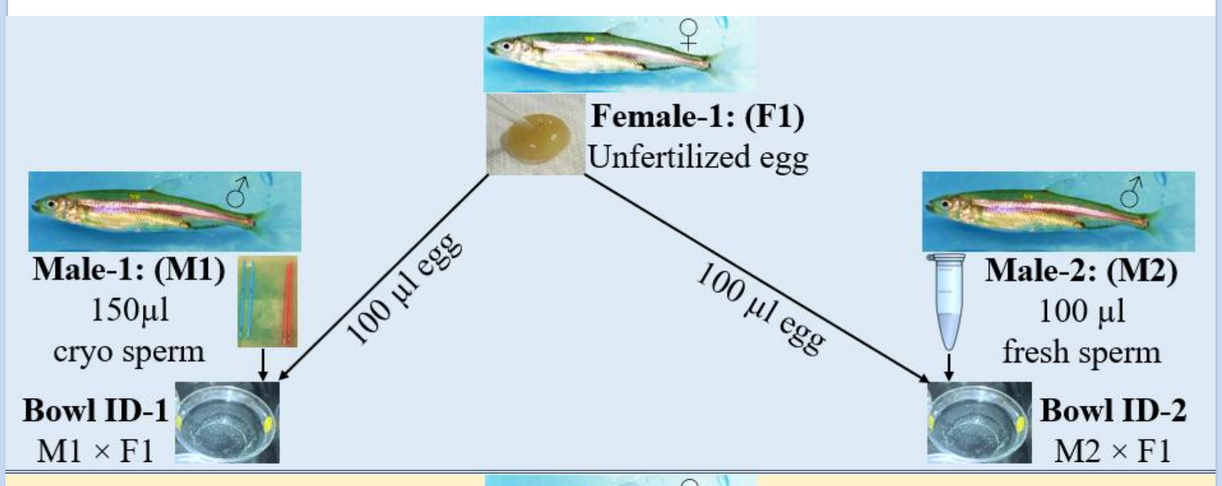

Figure 1. Partial crossing fertilization design comparing fresh and cryopreserved sperm from two males and two females.

RESULTS

- FREEZESOL and HBSS showed the highest sperm motility
- Methanol was the only cryoprotectant preserving sperm
- Fertilization was reduced with cryopreserved sperm
- Hatching rate and larval survival were unaffected

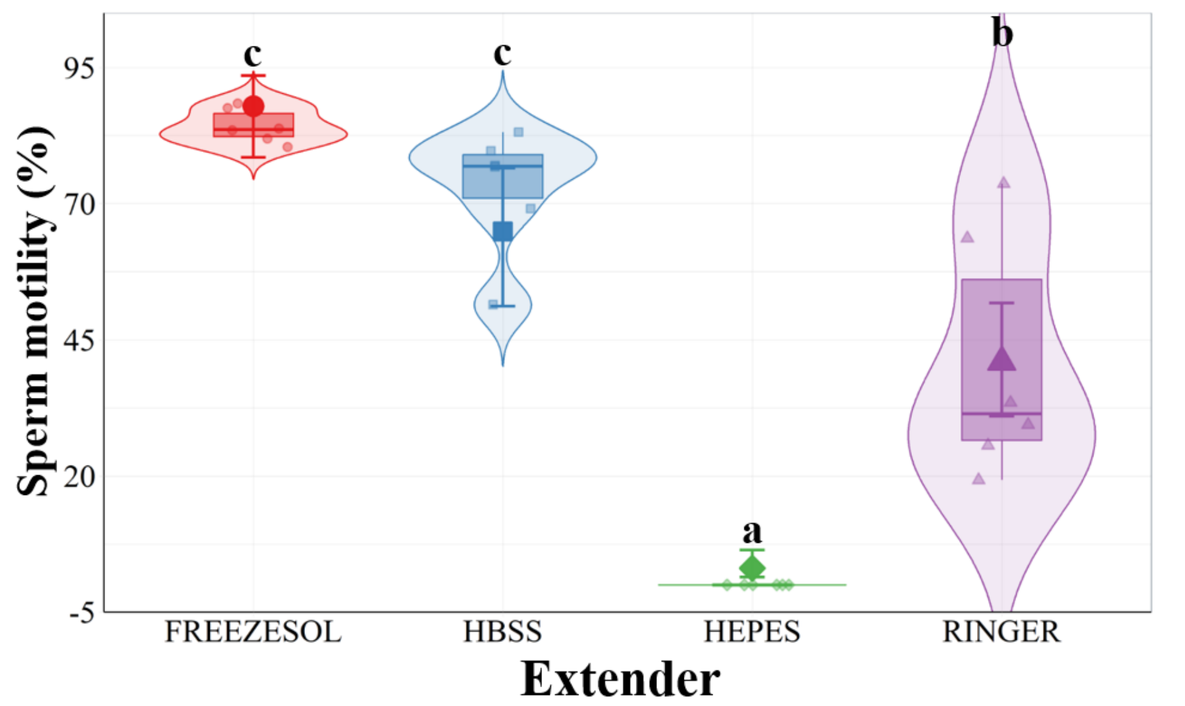

Figure 2. Variation in sperm motility (%) among four extenders.

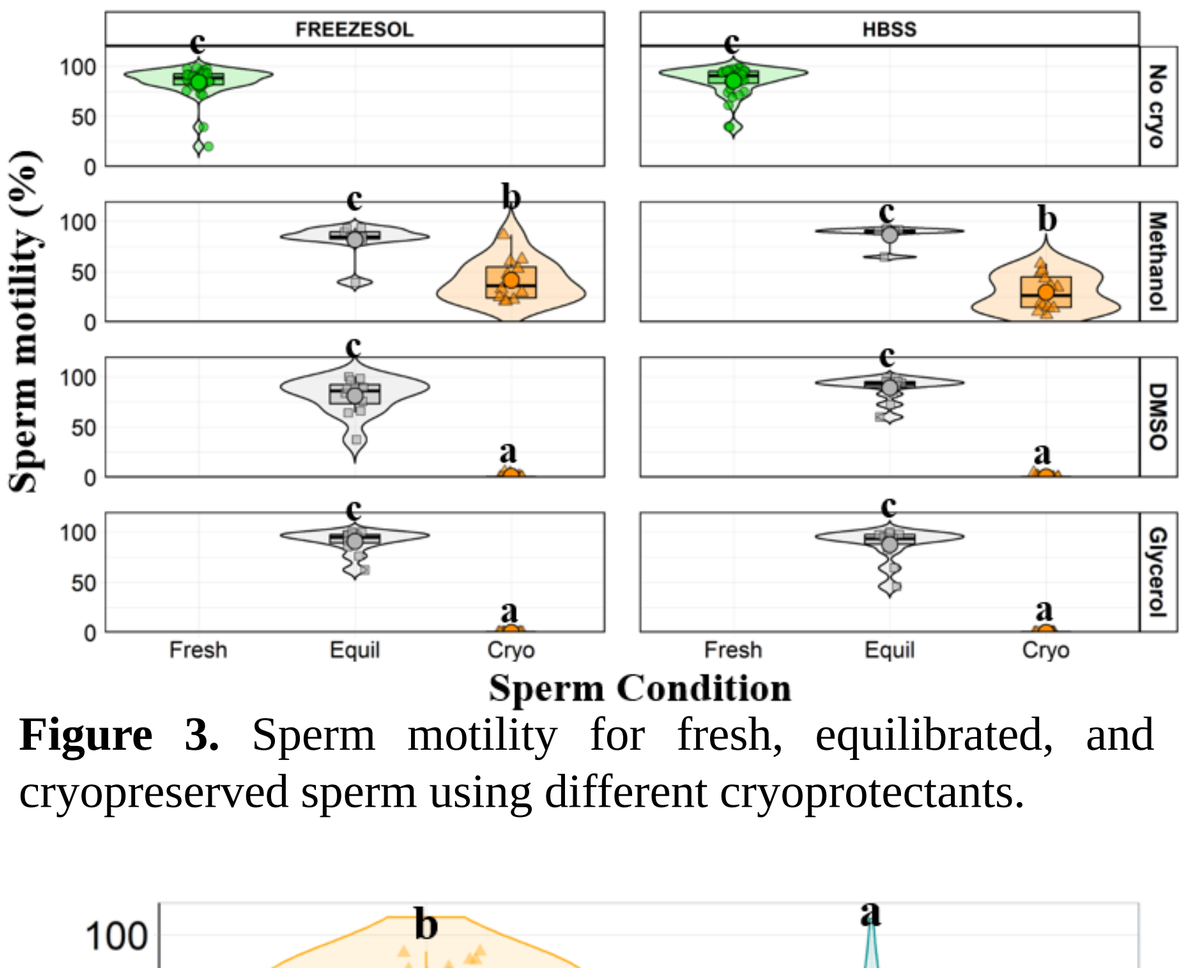

Figure 3. Sperm motility for fresh, equilibrated, and cryopreserved sperm using different cryoprotectants.

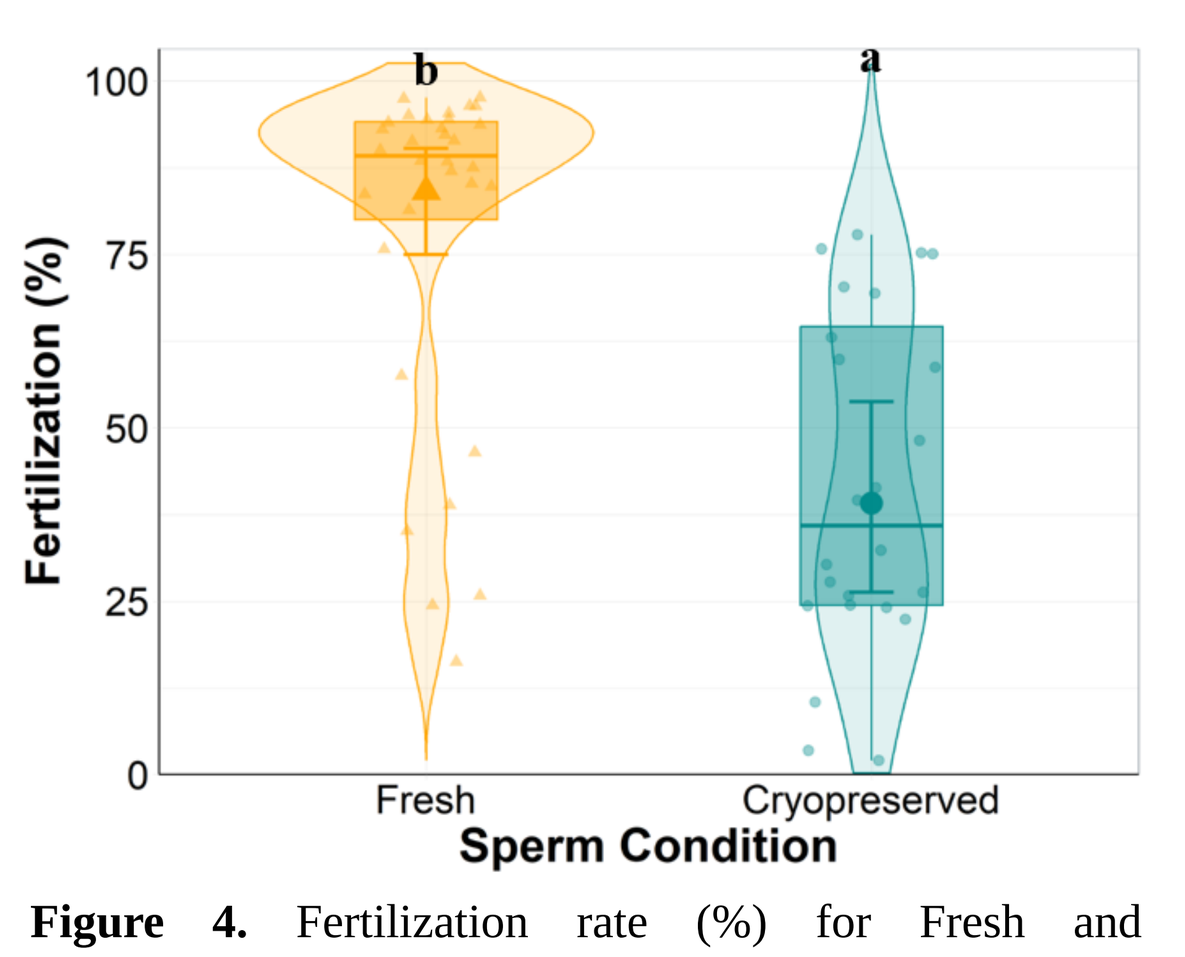

Figure 4. Fertilization rate (%) for Fresh and Cryopreserved sperm.

Post-fertilization. No differences were detected in hatching rate ($p = 0.11$) or larval survival ($p = 0.61$).

CONCLUSION

A protocol using FREEZESOL with 10% methanol preserves Delta Smelt sperm function and supports normal larval performance. This approach offers a practical tool for genetic resource banking and conservation hatchery operations.