

Studies on Cryopreservation of D-shaped and Umbo Larvae of Arkshell (*Scapharca broughtonii*)

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The present study examined the possibility of cryopreservation of the D-shaped and umbo larvae of arkshell (*Scapharca broughtonii*), in terms of the survival rates after freezing and thawing. D-shaped and umbo larvae of arkshells were obtained from a shellfish farming on Yosu city. The average shell lengths were $93.3 \pm 10.1 \mu\text{m}$ and $201.7 \pm 13.5 \mu\text{m}$, respectively. Five cryoprotectants (CPAs), dimethyl sulfoxide (DMSO), glycerol, ethylene glycol (EG), propylene glycol (PG), and methanol, were tested at the concentrations of 1.5, 2.0 and 2.5 M. After larvae suspended in CPAs, cryoprotectants were loaded in 0.5 ml straws at a larval density of 50~100 larvae per straw, and equilibrated for 10 and 20 minute at room temperature (23°C), respectively. Straws were cooled at a rate of 1°C/min from 0°C to -12°C, held for 5 min at -12°C, and then cooled at 2°C/min to -35°C and equilibrated for 5 min followed by plunging in liquid nitrogen. After storage in liquid nitrogen for 1 day, straws were thawed in a 30°C water. As soon as straws were observed to melt, larvae were diluted with an equal volume of ASW and then washed twice with a large volume of ASW at an interval of 2 min to unload the CPAs. The results showed that after equilibration for 10 and 20 minute at room temperature, no larvae survived using methanol as CPAs, and it was observed that larval shells all open slightly, and larval flesh broke down and slopped over the shells. The highest survival rates (D-shaped larvae: 77.6%, umbo larvae: 59.3%) were obtained with 2M DMSO, and 1.5M glycerol yielded survival rates of 53.8% for D-shaped larvae and 37.5% for umbo larvae. The surviving D-shaped larvae showed active rotary motion and perfect membrane integrity and cytoplasmic normality, and the vigorous movement of veliger cilia was observed inside the closed shells. The breakdown of tissue occurred in the abnormal larvae, and the isolated cell often run out of shells.

Key words) *Scapharca broughtonii*, *Cryopreservation*, *D-shaped larvae*, *Umbo larvae*