

**The Influence of Developmental Stages and Different Kinds and Concentrations of Protective Additives in Cryopreservation of Surf Clam (*Spisula Sachalinensis*) Larvae**

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This study was performed to find out the optimal larval stage among trochophore, D-shaped and umbo stage larvae and the desirable protective additive such as fructose, glucose, sucrose and trehalose with cryoprotectant for cryopreservation of surf clam, *Spisula sachalinensis* larvae. Dimethyl sulfoxide and ethylene glycol were used as cryoprotectant and each cryoprotectant was made to 2.0 M with previous protective additives. The larvae were immersed in the preparations waited for 15 minutes to reach equilibration, and then frozen in a program freezer (-35 °C) and liquid nitrogen (-196°C). The freezing rate of 1.0°C /min. was used for cryopreservation of trochophores before seeding temperature (-12 °C).

The survival rate of frozen-thawed larvae increased as larval developing and that of umbo stage larvae was the highest as  $96.1 \pm 1.0\%$ . The presence of lower concentration of disaccharides as sucrose or trehalose significantly enhanced survival rate when mixed with cryoprotectants ( $P < 0.05$ ).

The results of our study indicate that desirable developmental stages of larvae and protective additive for cryopreservation are the umbo stage larvae and 0.2 M sucrose, respectively.

Keywords) *Surf clam, Spisula sachalinensis, Developmental stage, Protective additive, Cryopreservation*