CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
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S1909

Project Title
Investigating Protocols for Haliotis rufescens Egg Cryopreservation

Abstract

Objectives/Goals
The number of aquatic species listed as endangered or threatened is growing. Cryopreservation may offer the opportunity to preserve genetic material in DNA banks, conduct genetic research, and optimize strategies to enhance survivability in the wild. The purpose of this study was to investigate cryopreservation protocols which would yield results for normal red abalone (Haliotis rufescens) egg phenotype after thawing, as a possible model for the at risk abalone species. The objectives were to determine whether eggs can be successfully cryopreserved using a stepwise cooling and thawing procedure, which cryoprotectant agent (CPA) is most effective, and whether a non-permeating cryoprotectant (sucrose) can aid in the thawing and rehydration process.

Methods/Materials
After induced spawning (with prepared H(2)O(2)/Tris Solution), egg collection and centrifuging, four freezing trials were conducted using CPA 'Freezing Medium'(FM) or dimethyl sulfoxide (DMSO) at 8 and 16 mins stepwise gradual cooling at temps 14, 4, -40 degrees C, and non-stepwise 1 min cooling, before plunging into liquid nitrogen. A total of 45 stepwise (5 to 7mins at temps -40, 4, 14 degrees C) and non-stepwise thawing protocols were tested with and without sucrose at 5g, 2.5g or 1.25g/80ml water.

Results
Best results were found in protocols using DMSO at 8 and 16 mins stepwise cooling, and stepwise thawing with sucrose amounts of 1.25g or 2.5g, yielding 25% to 50% (8mins) and 50% to 75% (16mins) normal egg phenotypes. Non-stepwise protocols resulted in 75% to 100% eggs bursting with the remainder abnormal. Thawing protocols without sucrose resulted in 100% eggs bursting. Protocols using FM resulted in 50% to 100% eggs bursting, with the remainder abnormal.

Conclusions/Discussion
Stepwise cooling and thawing, DMSO as CPA, and the use of sucrose in acting as a non-permeating buffer in diminishing effects of rapid rehydration, contribute to maintain normal egg phenotype after cryopreservation, which supports my hypothesis. However, it is unknown whether eggs were still viable. The intention was to verify the survivabilty of eggs with live sperm, but male spawning was unsuccessful. Further research will include in vitro fertilization studies, and hopefully cryogenic studies on the endangered white and threatened black abalone.

Summary Statement
This project was conducted to test which protocols would give best results for normal red abalone egg phenotype after cryopreservation.

Help Received
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