

## Effects of the slow cooling during cryopreservation on the survival and morphology of Taiwan shoveljaw carp (



Over the past decades, pollution, overfishing, and habitat degradation have driven the population size of Taiwan shoveljaw carp down markedly in Taiwan. Cryopreservation is a useful tool which could be used to maintain genetic resources to protect and preserve this endemic species. Four cryoprotectants [dimethyl sulphoxide (DMSO), dimethylacetamide (DMA), glycerol and methanol] and six freezing rates ( $0.5, 1, 2, 4, 8, 16 \text{ }^{\circ}\text{C min}^{-1}$ ) were tested in order to develop an optimal controlled slow-freezing protocol for Taiwan shoveljaw carp spermatozoa. Samples were subsequently examined under the scanning electron microscope to reveal whether cryopreservation had affected their ultrastructural morphology. The highest survival rate ( $50.1 \pm 2.0\%$ ) was observed with a freezing rate of  $8 \text{ }^{\circ}\text{C min}^{-1}$  in 1M DMSO, using SYBR-14 + PI staining. Fertility and hatching rate results using frozen-thawed spermatozoa ( $90.2 \pm 2.2\%$  and  $22.3 \pm 2.5\%$ , respectively) were not significantly different from results with fresh spermatozoa. After cryopreservation,  $21.0 \pm 1.6\%$  of frozen-thawed spermatozoa had mid-piece swelling and rupture of the head.

Cryopreservation might, therefore, slightly affect Taiwan shoveljaw carp spermatozoa in terms of morphological change. However, these alterations could be compensated by using large enough numbers of normally functioning frozen-thawed spermatozoa to achieve a standard equal to fresh spermatozoa. This is the first report of successful cryopreservation of Taiwan shoveljaw carp spermatozoa using a controlled slow-cooling method.

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