# SPERM CRYOPRESERVATION OF ENDEMIC FRESHWATER FISH *Systomus spilurus*.Sudasinghe 2018

K.M.N. Madusanka1, C.N. Walpita1\*, A.R.Mudalige2, A.R.S.B. Athauda 3

*1Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka*

*2National Aquaculture Development Center, Dambulla.*

*3Department of Animal Science, Faculty of Agriculture, University of Peradeniya*

*\*chaminda.walpita@gmail.com*

Mas pethiya (*Systomus spilurus*)is an endemic fish to Sri Lanka*.* Fishing pressure and other anthropogenic reasons significantly reduce their natural population.Sperm cryopreservation is considered as a method of preservation of fish species and opens a new avenue to conserve these species. In this study, an existing cryopreservation protocol developed for another fish species was followed for *S. spilurus.* After sperm was collected from healthy *S. spilurus* males, sperm quality was evaluated. The motility of *S. spilurus* brooderfresh semenwas observed as 91-100% under the microscope. The sperm cell density of *S. spilurus* is 30.69x109 cells ml-1 and the spermatocrit value of sperm is given as 60% ±1. Cryopreservation solution was prepared by using 65ml of extender and 15 ml of dimethyl sulphoxide mixed to prepare diluents, filled 3;1 spermatozoa were introduced to each cryovial and vials were kept in 8 ˚C -10 ˚C for an equilibration period of 45 minutes. Freezing was carried out at -15 ˚C in liquid nitrogen vapor for 3-4 minutes and frozen spermatozoa were kept in a goblet stored in liquid nitrogen at the -196 ˚ one-month time period. Frozen sperm were thawed at the temperature of 38±1 ̊C. Motility was observed under the microscope, for one week, two weeks, three weeks (71-90%), and four weeks (70%) after cryopreservation. The time of spermatozoa viability was observed at around 48 seconds after four weeks of cryopreservation and also 2 – 4 ml of milt volume per kg of body weight from *S. spilurus*. The fertilization rate of cryogenically frozen/thawed sperm had a 46% fertilization rate and 82% hatching rate*.* Considering the findings, cryopreservation success is marginal and this protocol could be optimized for *S. spilurus*.

**Keywords:** *Cell motility, Cell viability, Cryopreservation, Spermatozoa, Systomus spiluru*