CRYOPRESERVING EUROPEAN EEL (*Anguilla anguilla*) SPERM: A STEP TOWARDS A SUSTAINABLE CRYOBANK

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1 Introduction

The European eel (*Anguilla Anguilla*) is listed as 'critically endangered' on the IUCN Red List of Threatened Species.When artificially breeding this species, it is common to use wild females and farmed males. This is because the wild population is declining and the density is decreasing in the various basins, and they differentiate sexually by developing the female sex and adapting to the adverse conditions. In this regard, several research groups have experimented with **cryopreservation** of the seed in recent years for both Japonica (Tanaka et al.2002) and European eel (Müller et al. 2004; Asturiano et al. 2004).

Cryopreservation is an increasingly common practice in fish (Suquet et al.2020; Kopeika et al.2007). Cryopreservation of spermatozoa is a crucial method for conserving endangered species and maintaining biodiversity ex situ. This technique allows for the preservation of genetic material and the potential to restore original populations following environmental recovery. It is used in conservation programs, genetic improvement, and selective breeding of fish. **The study aimed to develop a technique for preserving *Anguilla anguilla* sperm to establish a national sperm bank**.

2 Material and Methods

Maturation

For this experiment, four wild males (mean weight: 128,63 gr ± 33,89 and average length: 143,10 gr ± 4,31) captured in November 2023 were induced following standard protocols by weekly injections with 1 IU/g BW hCG and started spermiation after a 12-week treatment.

Milt conservation

For each male, three ejaculates were collected on different 3 dates and treated as follows: semen samples were diluted in Tanaka's extender solution, ratio 1:8:1 semen/extender/methanol (Tanaka et al.2002) and working on refrigerate condition (4-6°C)

French straws conservation

The samples were packaged in French straws of 0,25 ml and frozen using nitrogen vapor by maintaining the straws at about 3 cm from the surface of the liquid nitrogen for 3 minutes.
The frozen straws were conserved in liquid nitrogen.

CASA analysis

To evaluate the effect of cryopreservation on sperm quality, each semen was analyzed for the determination of motility, before and after thawing, using both microscope and image analysis **CASA systems** (IVOS II – Hamilton Thorne).

3 Results and Discussion

The motility average value of fresh semen, before cryopreservation was **53±10,97%** while, after thawing was **22,45 ±7,95%,** (representing a yield upon thawing of 43,5±15,1%), with an activation time post-tawing of **35.5±3.8''**. The total number of straws obtained was 240 with a mean sperms' concentration of 2.600 x 10^6 /ml.

The graph in **Figure 1** shows the freezing yield of the eel semen for male (males 1-4) and date of collection and processing. In general, the freezing yield of the ejaculates collected the first day (05/04/24) showed a higher freezing yield compared to those of the other two dates, despite the same processing conditions and similar motilities of the fresh semen among ejaculates and dates. The reproductive period of the eels and the timing of the hormonal induction and semen collection might probably influence the quality of the semen.

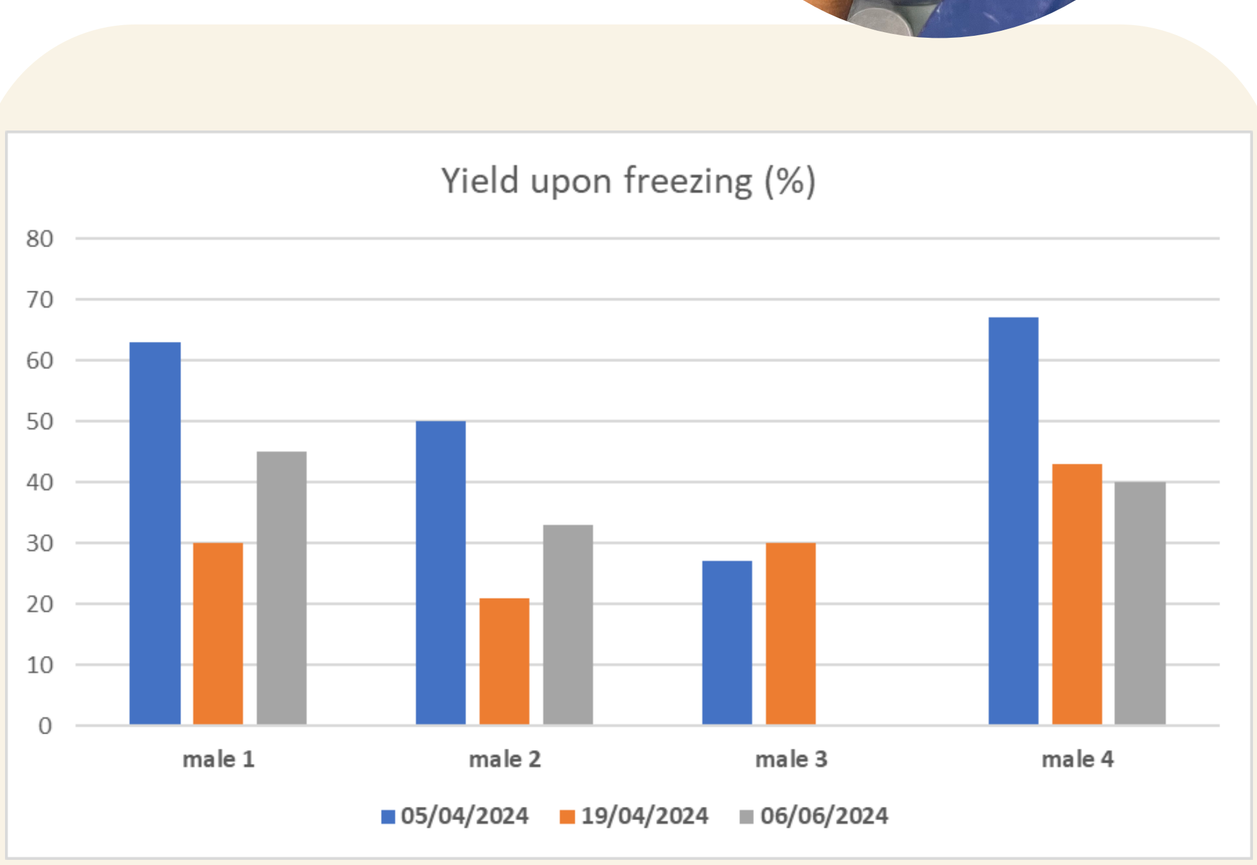

Figure 1: freezing yield of the semen for male (Males 1-4) and dates of collection and processing.

The yield upon thawing revealed very satisfying, despite the motility average values of the fresh semen, which resulted quite low. Further study will be focused on the application of cryopreserved sperm on eggs to perform fertilization trials, to confirm the sperm quality after thawing and the effectiveness freezing protocol for the sperm cryobank implementation.