



# Cryopreservation at the IEO

Workshop on Cryobanking in Aquatic Species

October 2<sup>nd</sup>, 2018

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# IEO Facilities



AQUA  
EXCEL  
2020



## MAP-IEO (Mazarrón Aquaculture facilities)

*Sperm bank of 42 European seabass (*Dicentrarchus labrax*) males from a Mediterranean population*



Sperm was collected from mature ♂ during natural spawning season by abdominal massage.

*Bol. Inst. Esp. Oceanogr. 21 (1-4). 2005: 213-217*

## Procedure

Sperm is collected in sterile individual plastic syringes (5 ml) without needle, and kept on crushed ice until its use

Diluted to a ratio of 1:1 in Mounib extender solution as modified by Legendre & Billard (1980)

Cryoprotectants 10% BSA and 10% DMSO

Diluted semen is packaged in 0.5 mL straws or 2 mL cryotubes and left to equilibrate for 30 min at 4°C.

Following equilibration, the straws and cryotubes are exposed to liquid nitrogen vapor for 10 or 20 min and plunged into liquid nitrogen (-196°C)

# Sperm quality assessment

## Sperm motility activation

### A two-step procedure

sperm is first diluted in the immobilizing solution (Ringer 200 mOsm/l) at 1:5.

Activation by mixing spermatozoa directly under the microscope with an activating solution (seawater, 1080mOsm/l) at 1:20.

## Sperm motility assessment

The sperm quality parameter used is the motility percentage

Motility was assessed by microscopic observation (400x)

### Sperm quality threshold

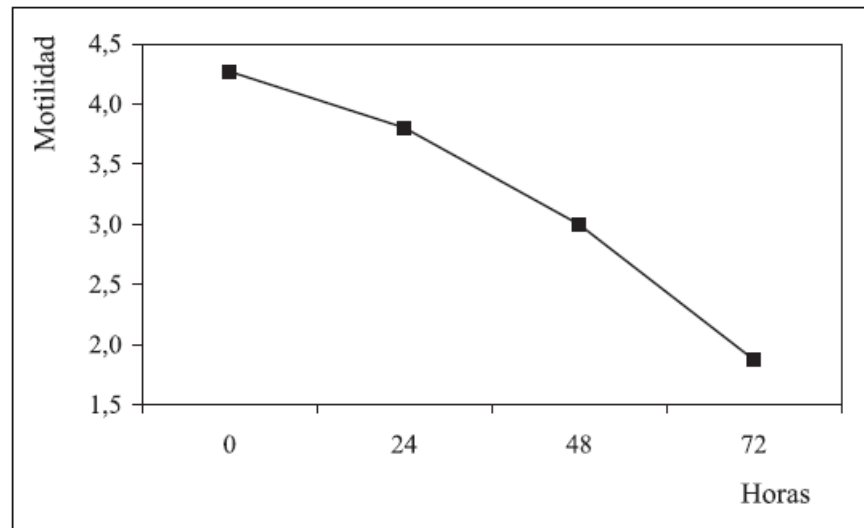
high and progressive motility (3 or more in Sánchez-Rodríguez (1975) scale) = cryopreservation



# Sperm thawing procedure

Straws: water bath at 40 °C for 7s.

Cryotubes: At 70 °C for 1.5 min.



Ringer 200 mOsm, 4°C





# AQUA EXCEL 2020

## Thank You!

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