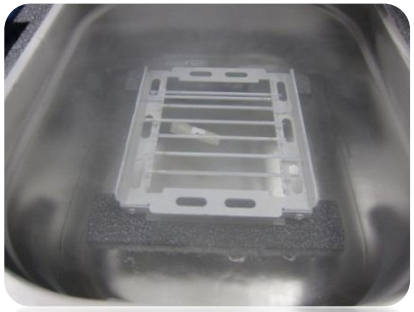
CRYOPRESERVATION AS A TOOL TO IMPROVE PERFORMANCE OF REPRODUCTION

Lilongwe, March 13th 2019

Dr. Sebastian Rakers



Fraunhofer EMB

What means Cryopreservation?

Fraunhofer EMB

WHY CRYOPRESERVATION?

Cryobiology and aquaculture?

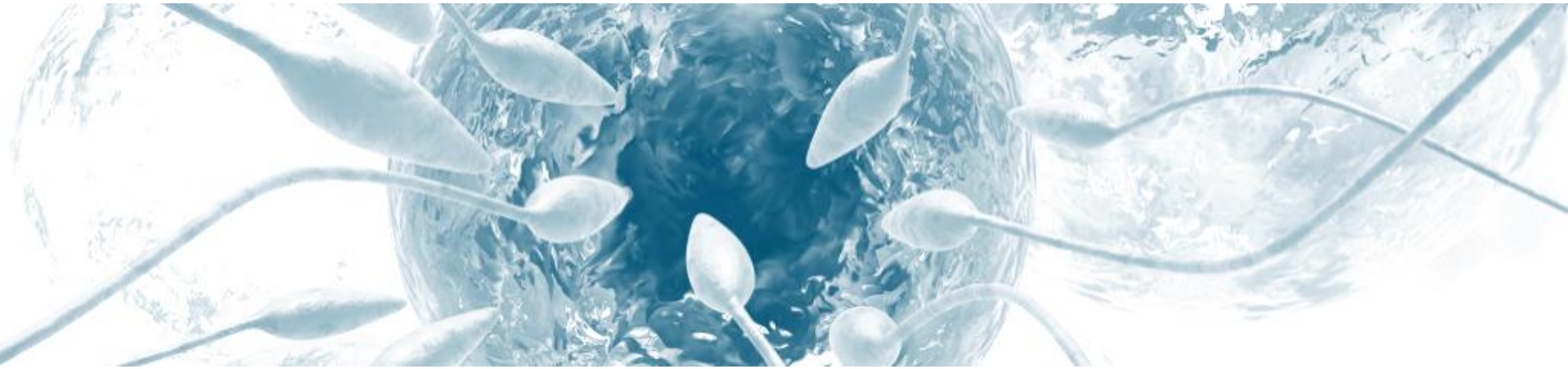
Management of reproduction

- ✓ Use of frozen sperm for handling routine in species with artificial fertilization
- ✓ Mating schemes independent of: maturation period, breeders availability, etc.
- ✓ Transport of gametes or embryos between farms instead of breeders
- ✓ Marketing of well-characterized and standard quality sperm

Genetic resource banking

- ✓ preservation of selected animals or stocks protected from outbreaks, catastrophes and genetic drift
- ✓ Preservation of biodiversity
- ✓ preservation of the genetics of valuable strains created for research or commercial production (polyploids, transgenics, etc)

CRYOPRESERVATION OF CHAMBO – WHERE TO START?



Cryogenic preservation protocols for over 200 species achieved

There is no standard fish gamete cryopreservation protocol available

We need to try different protocols (e.g. Review protocols for the successful preservation of closely related species)

HOW TO DO CRYOPRESERVATION?

Step 1: Select *O. karongae* broodstock

Step 2: bring selected *O. karongae* males to wet lab

HOW TO DO CRYOPRESERVATION?

Step 3: Anaesthetize fish

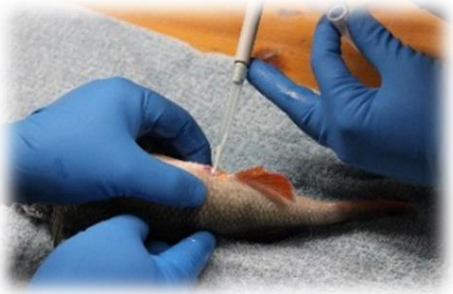
Step 4: Measure length and weight of individuals

anaesthesation bath: clove oil (1 ml/10 L) or benzocaine (100 mg/L)

HOW TO DO CRYOPRESERVATION?

Step 5: Collect semen, either by stripping or by dissection

Male Chambo showing the genital openings.



Collection of sperm.

Female Tilapia with eggs in mouth, collection of eggs into gauze nets

HOW TO DO CRYOPRESERVATION?

Step 6: Mix semen with pre-prepared extender and cryoprotectant

An extender is a solution consisting of inorganic and organic chemicals resembling that of blood or seminal plasma

Cryoprotectant + extender makes what we call ***diluent***

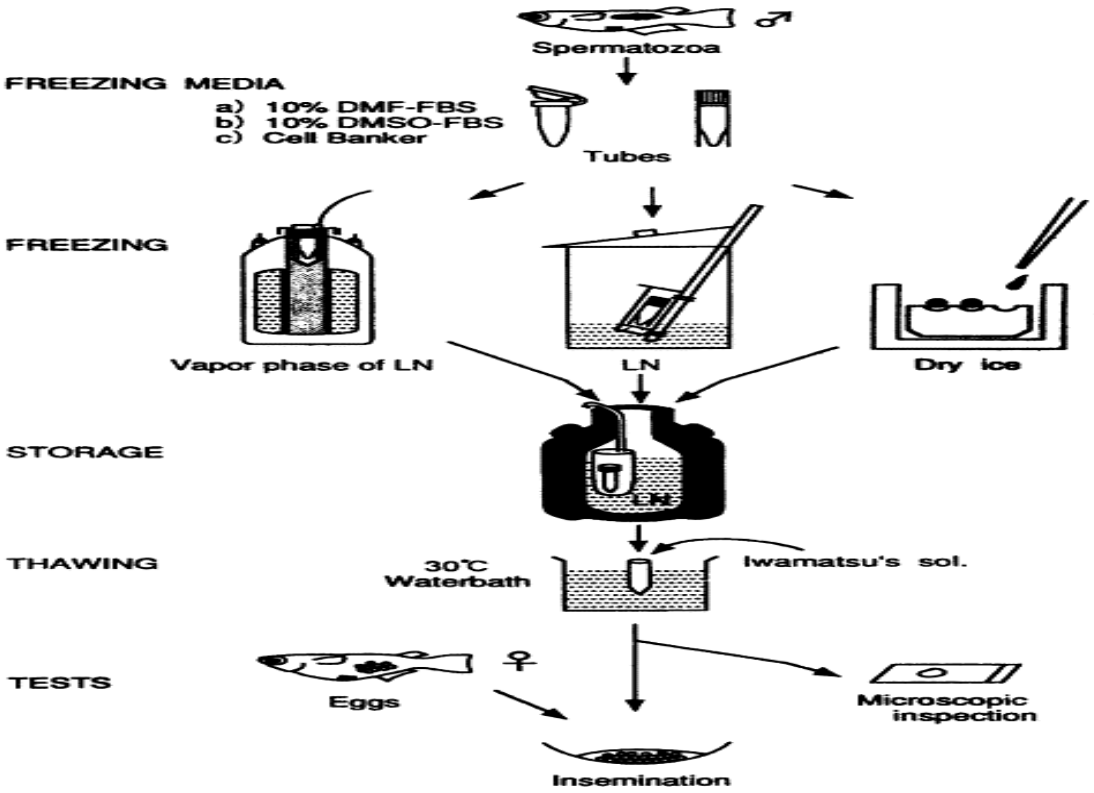
An example of extender is:

300 mM Sucrose
30 mM Tris (pH 8.0)

Then you can have diluent as:
Extender A (90ml) and DMSO (10ml)

HOW TO DO CRYOPRESERVATION?

Step 7: Fill storage containers with samples and freeze



Fraunhofer EMB

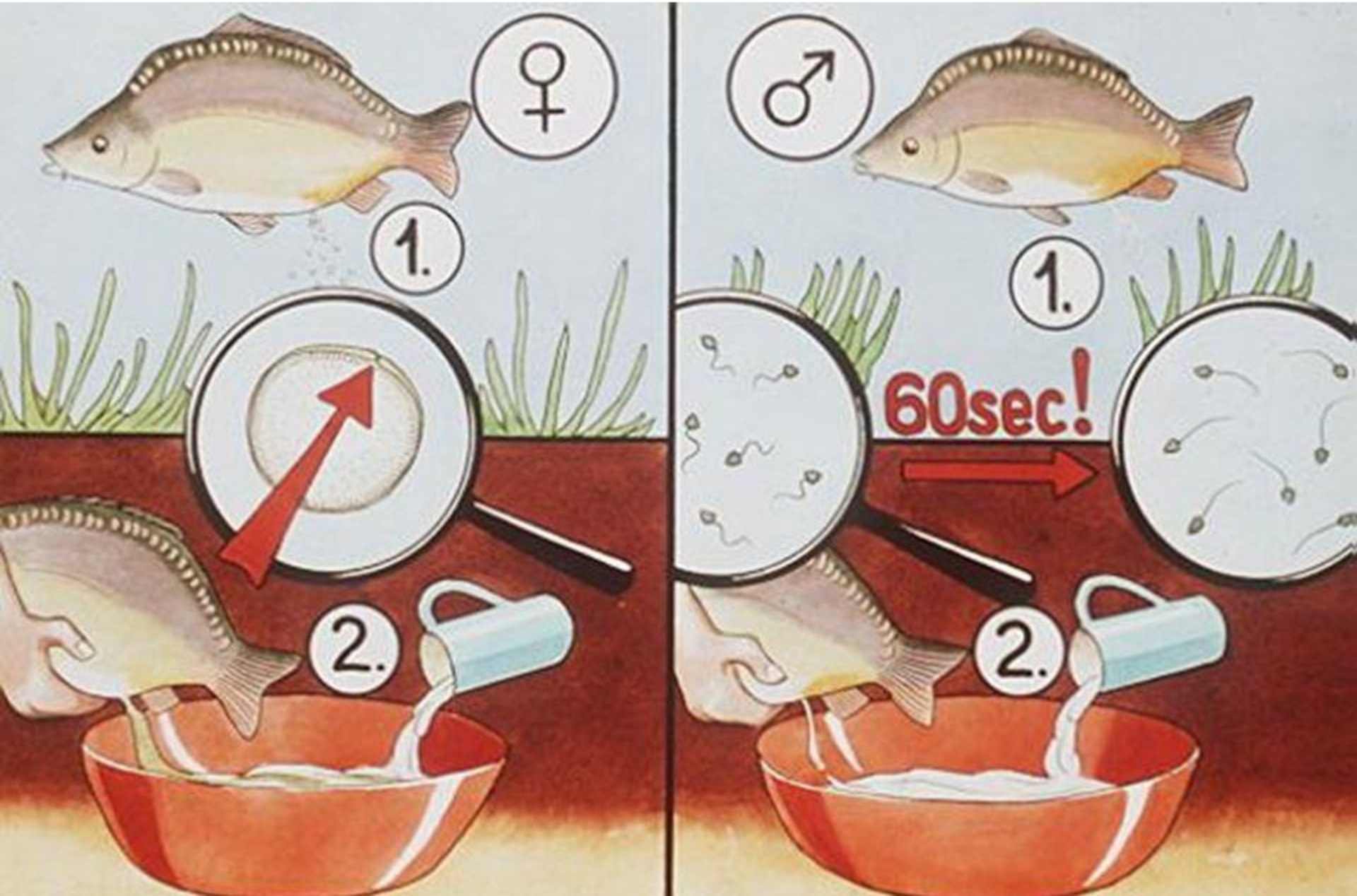
HOW TO DO CRYOPRESERVATION?

Step 8: Store samples in liquid nitrogen

Samples can be stored for years, if liquid nitrogen is refilled regularly!

Step 9: Thaw samples in water bath and fertilize eggs

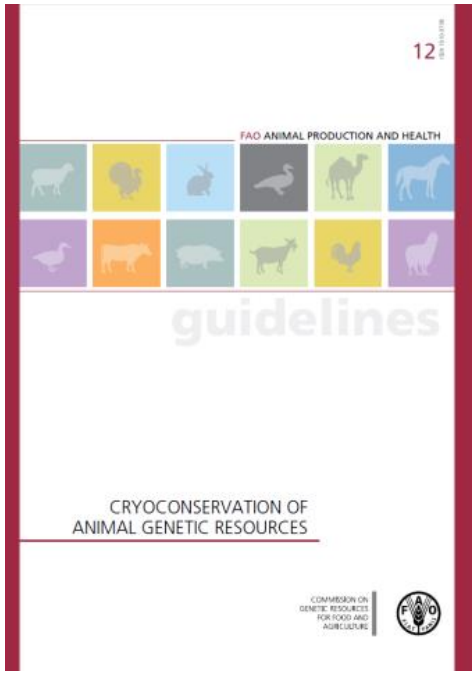
Artificial fertilization under dry conditions



AFTER THAWING?

Step 10: Place fertilized eggs into McDonalds jarr → hatchery procedure!

PRESERVATION OF AQUATIC GENETIC RESSOURCES: FISH ON REQUEST FROM BUNDA?!



Cryo-Brehm
Deutsche Zellbank für Wildtiere "Alfred Brehm"

Ich liebe Fisch
www.fish-for-life.org

Fraunhofer EMB

CRYOPRESERVATION - SUMMARY

- ✓ Cryopreservation is a feasible tool in aquaculture development
- ✓ Advantages of cryopreservation technology for management of fish reproduction and genetic resource banking
- ✓ Further adoption of technologies in Malawi and other African countries needed, challenges occur due to
  - ✓ Limited technical capacity,
  - ✓ Availability and supply of liquid nitrogen and
  - ✓ Power outages
- ✓ With cryopreservation technique, BUNDA has become a unique feature!

THANK YOU! DON'T GET FROZEN ;)

Fraunhofer
EMB