First Research Results of Perch Sperm Cryopreservation at the Research Facility Born (Germany)-A Pilot Experiment

EPFC
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• Research in the aquaculture sector $\rightarrow$ transfer to production/practical sektor

• Main purpose:
  1) converting science in **usable practice** and
  2) **to establish aquaculture operations** in Mecklenburg-Vorpommern

• support aquaculture operations
Location
The research facility
Working group **Aquatic Cell Technology & Aquaculture:**

1. Integrated aquaculture systems → Development of IMTA, Aquaponics

1. Aquatic biotechnology → application of cytological methods for aquaculture
   - 1. Fish health
   - 2. Reproduction

1. „Cryo Brehm“ → archive of cell cultures, e.g. different fish species

Cryopreservation of aquatic genetic resources: Foundation of an extended cell bank for carp strains (2012-2015)
Cryopreservation in short...

• Studies about the survival of live cell and tissues at extreme low temperatures

• Applied to the storage of e.g. sperm, oocytes and embryos

• The key is to:
  1.) Freeze cells without the formation of ice crystals → use of different solutions and chemicals to prevent this
  2.) to revive these cells after thawing

• Commonly used in animal reproduction: e.g. cattle, pigs, some fish
Optimization of the fingerling production of Eurasian perch (*Perca fluviatilis*) in the federal state Mecklenburg-Vorpommern

1. Develop a broodstock
2. Improve the reproduction of perch
3. Optimization of larval rearing and handling
First thoughts about using cryopreservation…

17.10.2017

Frederik Buhrke, Institute of Fisheries and Aquaculture
What do we want to achieve?

- Save tank capacity
- Provide late ripening females with high quality sperm
- Conserve our broodstock
- Testing different cryopreservation protocols
- Exchange with other broodstocks in Europe

First trials with cryopreservation
First steps of cryopreservation…

- From the literature, more than 200 fish species (e.g. carp, salmonids, catfish, whitefish, pike, milkfish, cod, zebrafish) can be cryopreserved

- → we have some experts for the cryopreservation of fish sperm in the room today


**Trials:**
- spring 2016
- off season autumn 2016
- spring 2017

**Main questions:**
- Test different methods?
- Which extenders should be used?
Material and methods

Spring 2017:

- 3-4 year old males

- N= 20, weight: 376g ± 86.5g
  TL= 30cm ± 3.3cm

- Ponds and FS, pH: 7.8, T= 13°C,
  95% oxygen saturation

- Natural spawners

- Protocols: Bernath 2013/2015, Irawan 2010
Sperm collection

• Anaesthetized the males
• Weight in g, TL in cm
• Collecting sperm with Eppendorf pipette
• Transferred in Eppendorf-Tubes (1.5ml)
• Storing on ice for 20 min
Sample processing in mobile laboratory

- Fully equipped laboratory in a self-unloading container (14 m²), 4 x 4 truck (300 HS) can reach almost every location

  Advantage: samples can be processed immediately

- Exemplary identification of sperm density with one sample: $1.832 \times 10^{10}$ sperm/ml

- Estimation of sperm motility (of fresh samples) (Zeiss Stereo Discovery V.20)
Cryopreservation I

- Addition of carp-Extender (Sucrose, NaCl, NaOH; 1:1) (Irawan et al. 2010), or Tanaka-Extender (NaCl, NaHCO₃;1:10) (Bernath et al. 2015)

- Stepwise addition of the cryoprotectants Dimethylsulfoxide (DMSO) (10 %) or Methanol (10 %)

- After equilibration all samples were transferred in 0.5 ml cryostraws, sealed (Minitube) and stored on ice
• frozen 3 cm above fluid nitrogen for 3 min on a floating frame and dumped in fluid nitrogen (3 min) (- 178 °C).

• The samples were stored in a LN-storage tank for **several days or longer**, until they have been used for thawing experiments.
• Samples were thawed for 10 sec in a 40 °C water bath
• Activity as well as motility tests were carried out after addition of Lahnsteiner-activation solution (Lahnsteiner et al., 2011) (Nikon ti eclipse)
• CASA analysis by free-ware ImageJ program
## Results

<table>
<thead>
<tr>
<th>Male</th>
<th>spawning</th>
<th>extender</th>
<th>motility after thawing in (min:s)</th>
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- All samples were motile for > 5 min before addition of cryoprotectants
- No motility observed in Tanaka-samples
- Motility was only observed in Irawan-samples
Results

- 4211 sperms marked and movement tracked
- More than 50% were activated
- But only 12% were motile
- VCL = 558.9 µm/s
- VSL = 333.2 µm/s
- Wobbling index = 47%
Fertilization

- Eggs post fertilization
- Fertilization with cryopreserved sperm (off season, natural season)
- Fertilization rate up to 50%
- No further development until hatch 😞
Summary

• Motility in samples was found with Carp-Extender and not with Tanaka-extender

• Highest motilities after thawing were up to 70 sec

• For the first time successful fertilization with cryopreserved sperm in our facility

• First samples further analyzed with CASA, needs to be optimized
Outlook
Discussion

• Carp-Extender (Irawan et al., 2010)
  - activatable
  - post-thaw motility
  - fertilization

• Tanaka-Extender (Bernath et al., 2015)
  - Not activatable
  - no post-thaw motility

BUT: Works in other experiments! e.g. Bernath et al. (2015), Kása et al. 2016

Obvious mistakes?

How long can you store sperm with Tanaka-Extender?

Slow-equilibrium programmable freezing? (Kása et al. 2016)

Ultrarapid non-equilibrium freezing? → which is the way to go?
Thank you for your attention.

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