Recent advances in cryopreservation od salmonid fish semen

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Justification for the studies

Poor performance of published protocols, low post-thaw quality of semen and a very short recommended time for fertilization (30 s)

Promising preliminary results indicating good post-thaw rainbow trout sperm quality with the use of glucose-methanol extender.

Justification for the study

Low quality of cryopreserved semen.

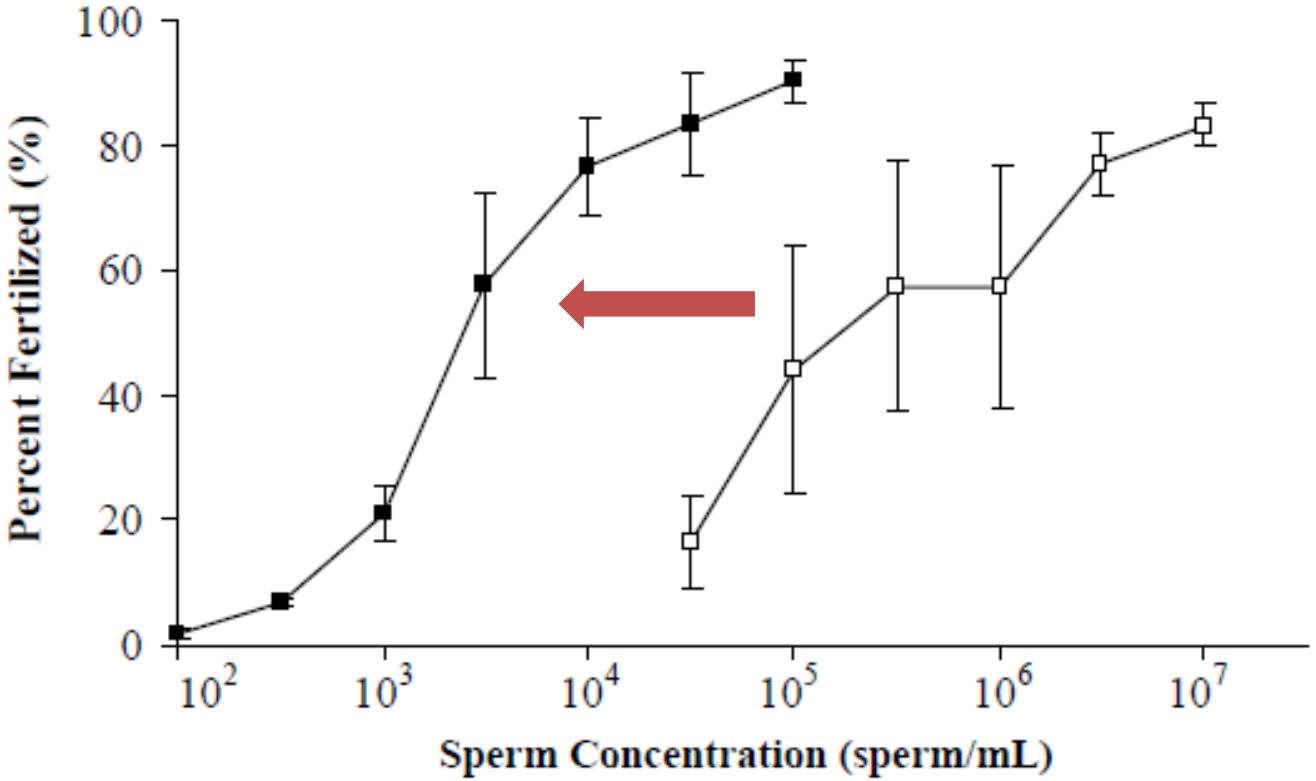
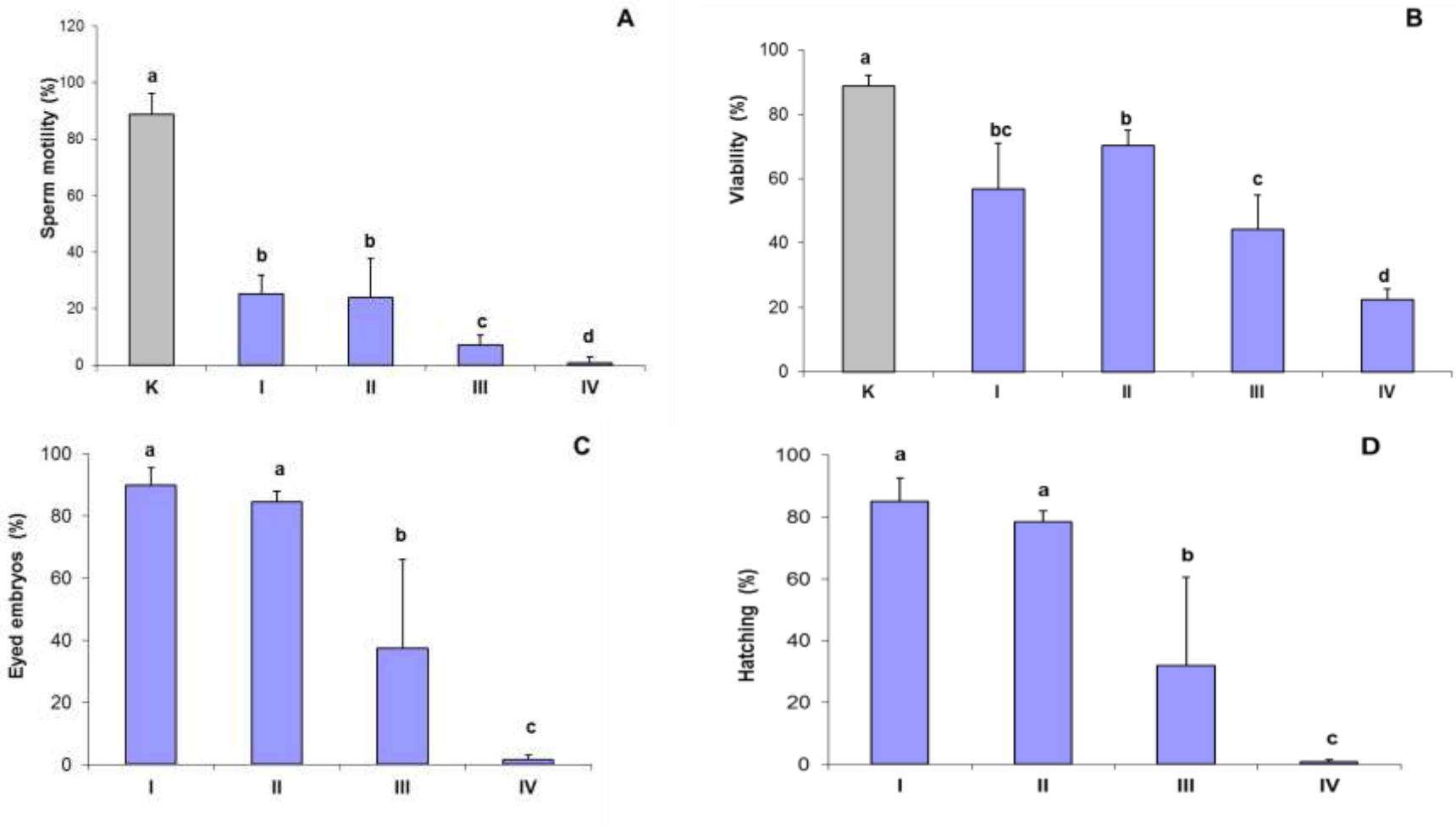


Figure 3. Fertilization (mean ± SEM) of eggs using fresh (closed squares) or cryopreserved (open squares) Pacific oyster sperm. Three pools of sperm were collected, cryopreserved and assayed independently. The same batches of eggs were used with the fresh and thawed sperm. (Modified from Adams et al. 2004).

Adams, S. L., J. F. Smith, R. Tervit, L. T. McGowan, R. D. Roberts, A. R. Janke, N. G. King, S. L. Gale and S. C. Webb. 2011. Cryopreservation of Molluscan Sperm: Pacific Oyster, Green-lipped Mussel, and Paua Abalone. In: *Cryopreservation in Aquatic Species,* 2nd Edition. T. R. Tiersch and C. C. Green, editors. World Aquaculture Society, Baton Rouge, Louisiana. Pp. 562-573.

Cryopreservation of rainbow trout semen using four different extenders

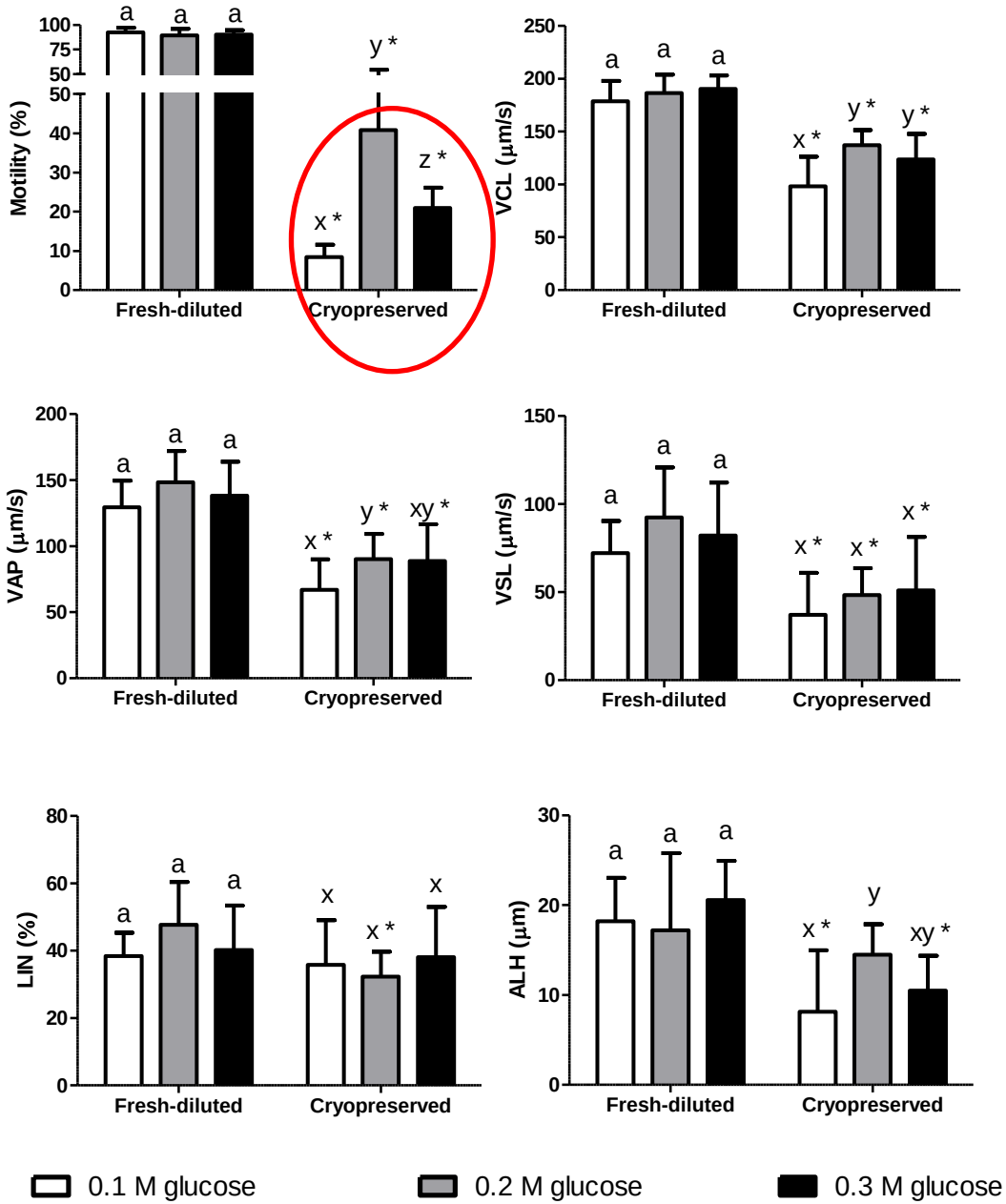


I 0.3 M glucose, 10% methanol; II 0.3 M glucose, 10% DMSO

Methanol – permeating cryoprotectant

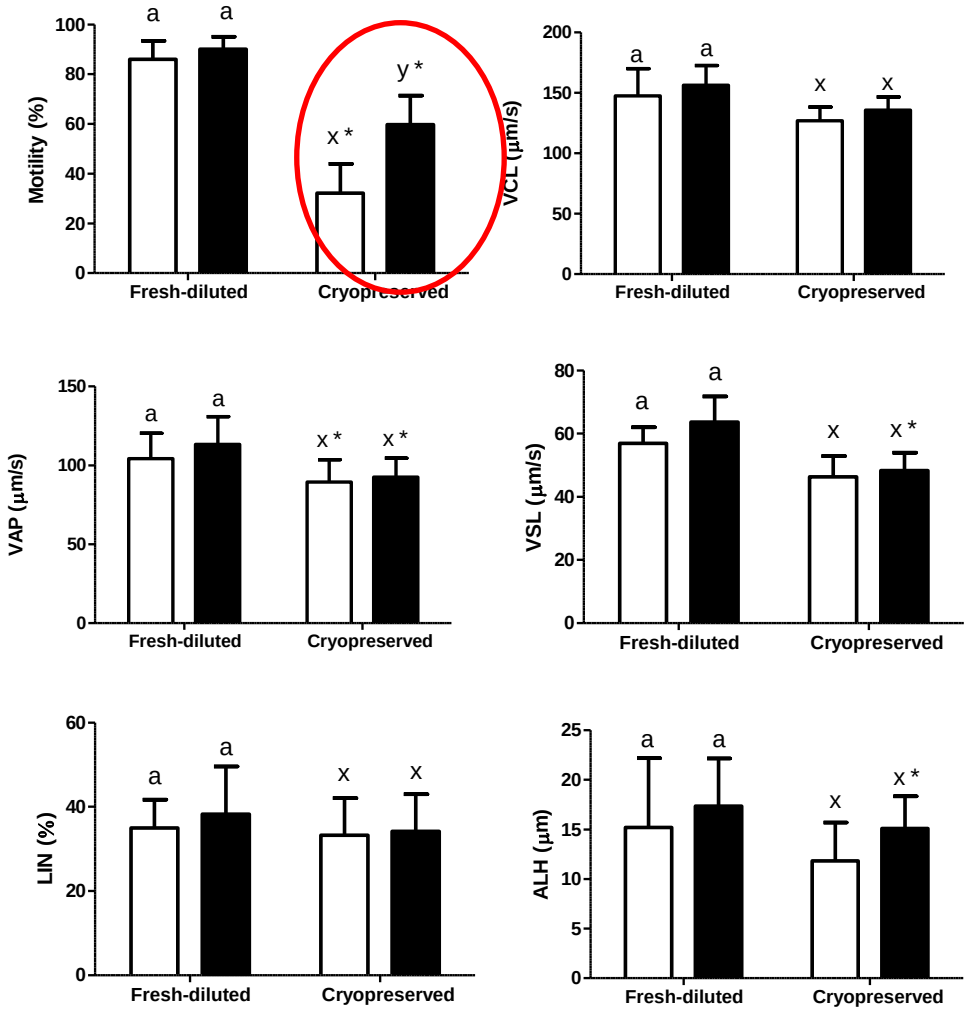
Glucose – nonpermeating cryoprotectant

Glucose concentration in the extender is important for the cryopreservation of rainbow trout semen

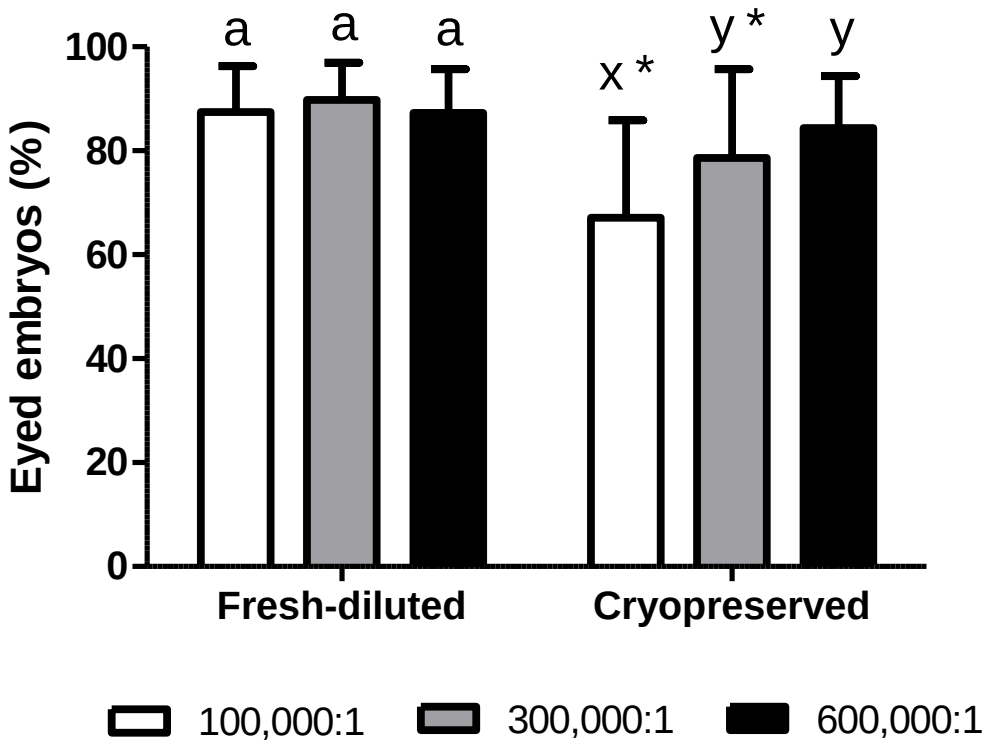


Dilution: 1:3

Effects of 1:3 and 1:5 sperm-to-extender dilution ratios on sperm motility parameters of fresh and cryopreserved semen



Sperm motility characteristics and fertilization rates of fresh and cryopreserved semen



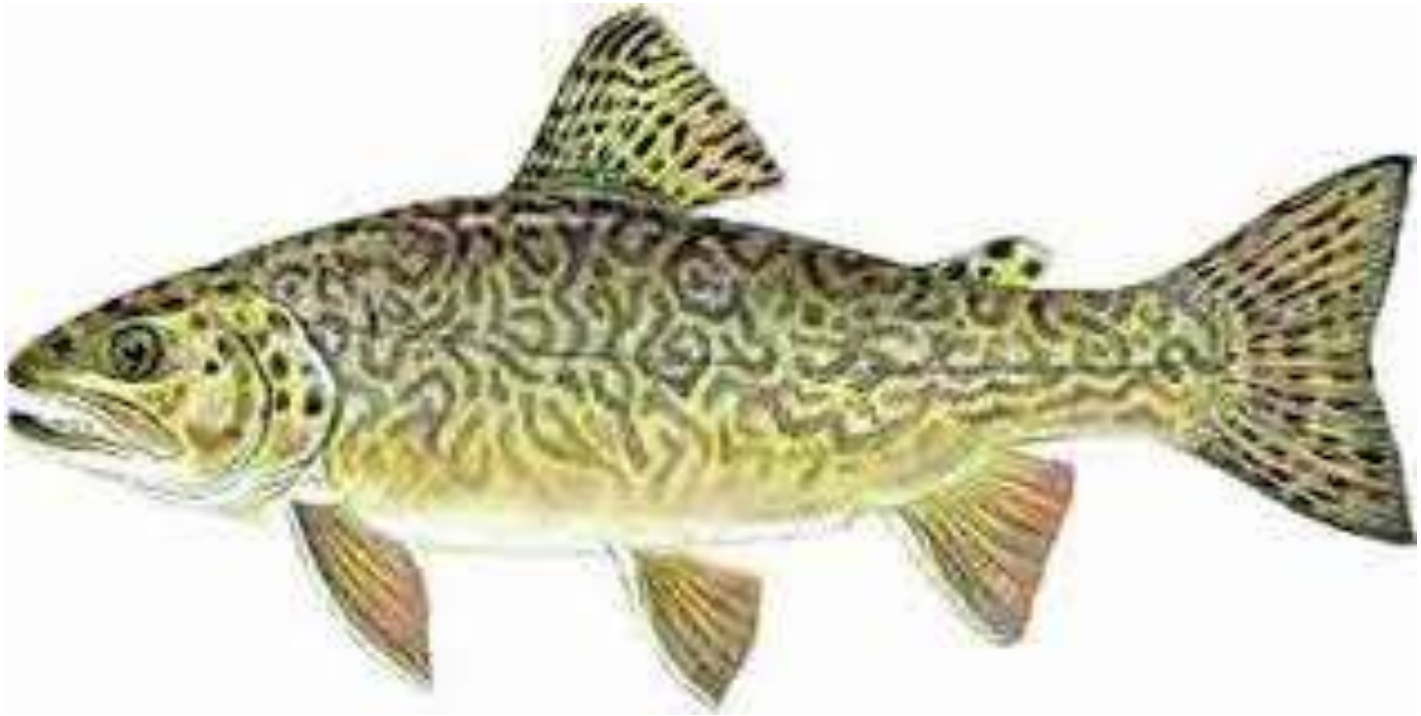
Post-thaw motility – 49.9 ± 6.8%

Mean sperm concentration and osmolality of fresh undiluted semen were 10.87 ± 2.48 x 10^9 spermatozoa and 251 ± 39 mOsm/kg, respectively.

Brown trout (*Salmo trutta* m. *fario* L.)

- a major source of freshwater fish resources in Europe because of its commercial value for aquaculture and extreme importance for angling.
- naturally subdivided into a large number of reproductively isolated and genetically distinct populations

The **tiger trout** (*Salmo trutta* X *Salvelinus fontinalis*) is a sterile, intergeneric hybrid of the brown trout (*Salmo trutta*) and the brook trout (*Salvelinus fontinalis*).



www.utahfishfinder.com/graphics/tiger_trout.jpg

Brook trout (*Salvelinus fontinalis*; Mitchill)

Important commercially, recreationally, and ecologically in Europe. It is of interest in aquaculture because it is almost completely resistant to the viral hemorrhagic septicemia virus and can easily be subjected to genome manipulation.

Sparctic Char: Strange Nighttime Saltwater Spawners from Europe!

December 3, 2011 By JD — 4 Comments

Spawning pair of Sparctic Char in Holland's Lake Oostvoorne (Janny Bosman photo)

http://www.fishwithjd.com/2011/12/03/se/

Materials and Methods

Source of milt

Brown trout (n=9); 3 years of age
Brook trout (n=9; 2 years of age

Cryopreservation

0.2M glucose in 9% MeOH;
Dilution 1:5 in 0.25 ml straws;
15 min equilibration,
Thawing 40ºC, 5 s.

Fertilization

Brown trout 1; 3; 6×10^5 sperm/egg ratio
Brook trout 3; 6×10^5 sperm/egg ratio
Fertilization rates were measured at the eyed and hatched stages.

Measurements of sperm motility and concentration

Sperm motility - measured in fresh semen after dilution and in frozen samples.
Sperm concentration - measured using Nucleocounter SP-100.

Sperm collection using a catheter

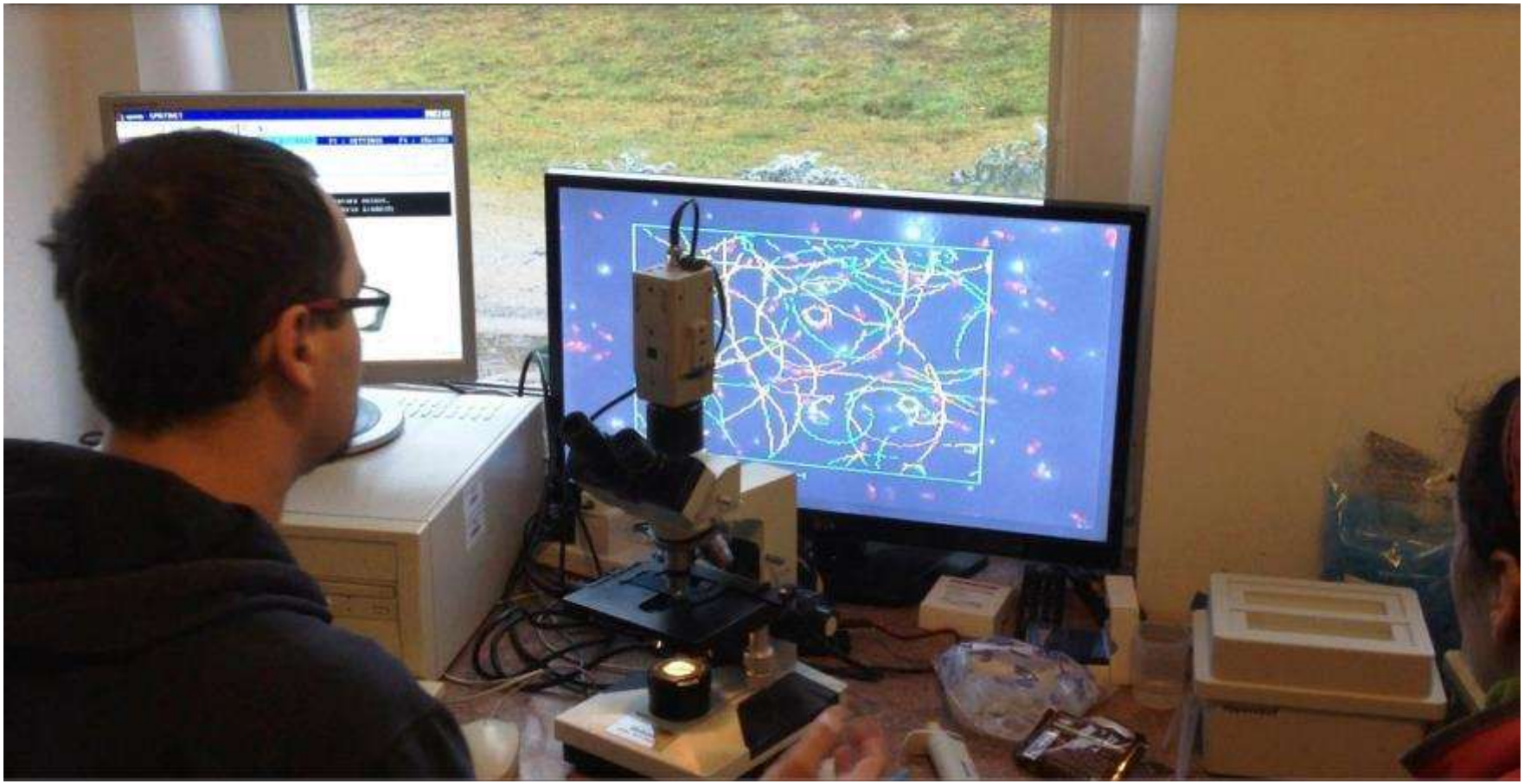
Collection of whitefish

European huchen

Reproductive system of sex-reversed females of rainbow trout


2

CASA analysis of sperm motility



Extension of semen with glucose-methanol extender

Equilibration of straws filled with extended semen

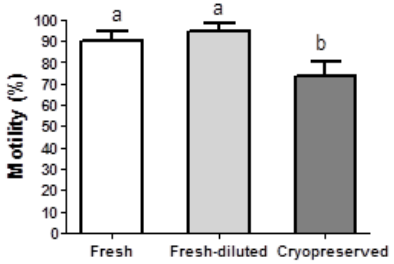
Equilibration of straws filled with extended semen

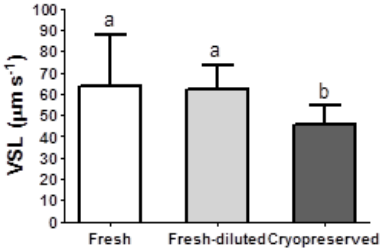
Freezing

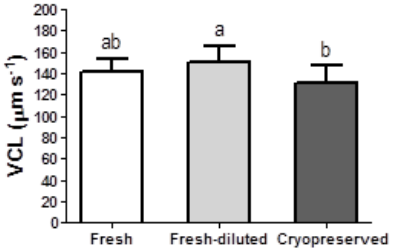
Thawing
40 °C, 5 s

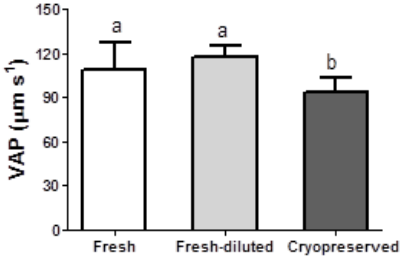
Effect of cryopreservation on sperm motility parameters

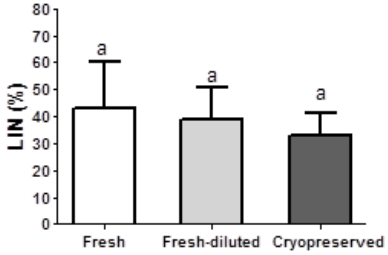
Brown trout

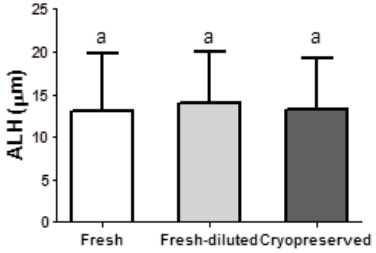




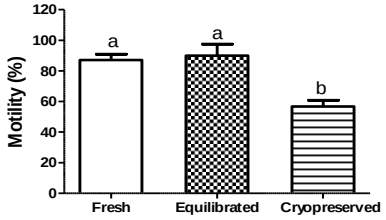


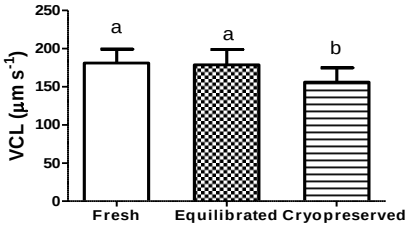


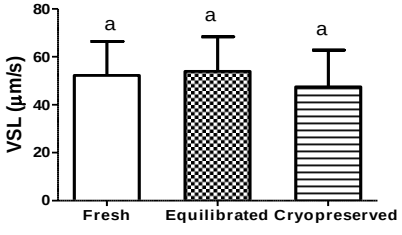


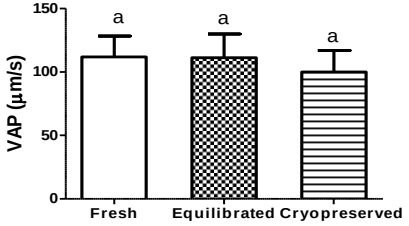


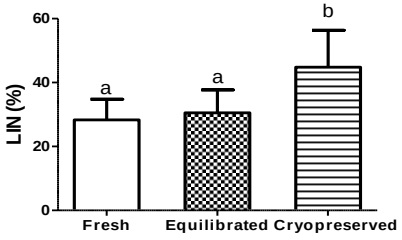
Brook trout

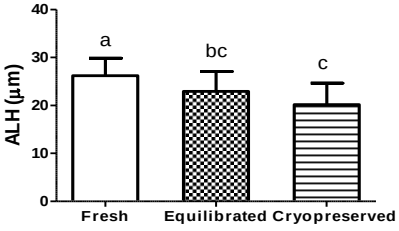






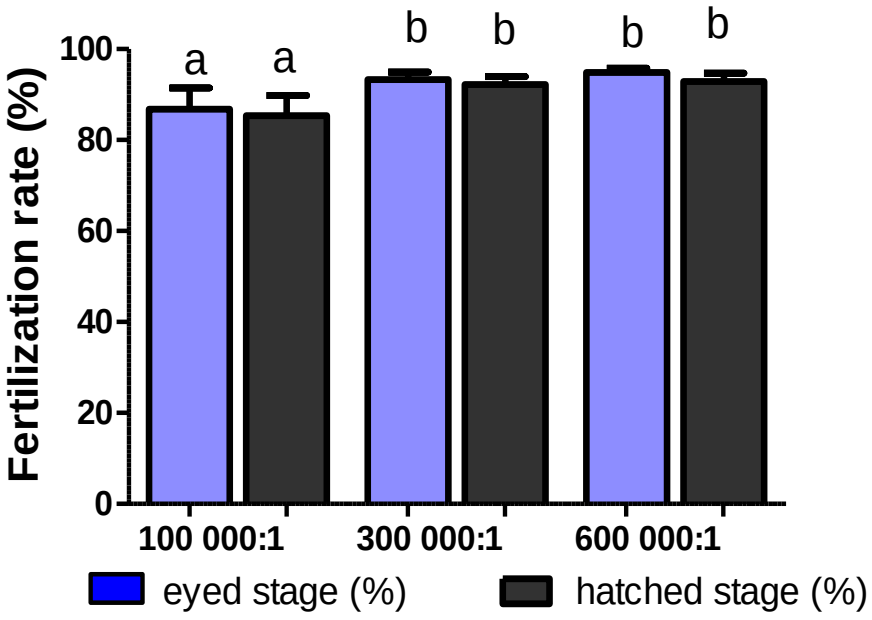




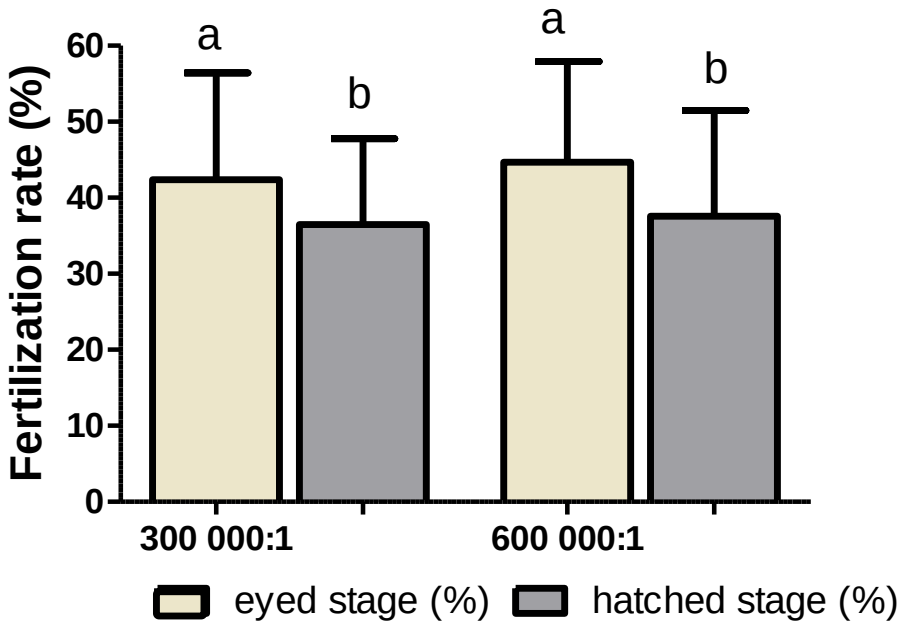


Effect of sperm-to-egg ratio on fertility of post-thaw cryopreserved sperm

Brown trout

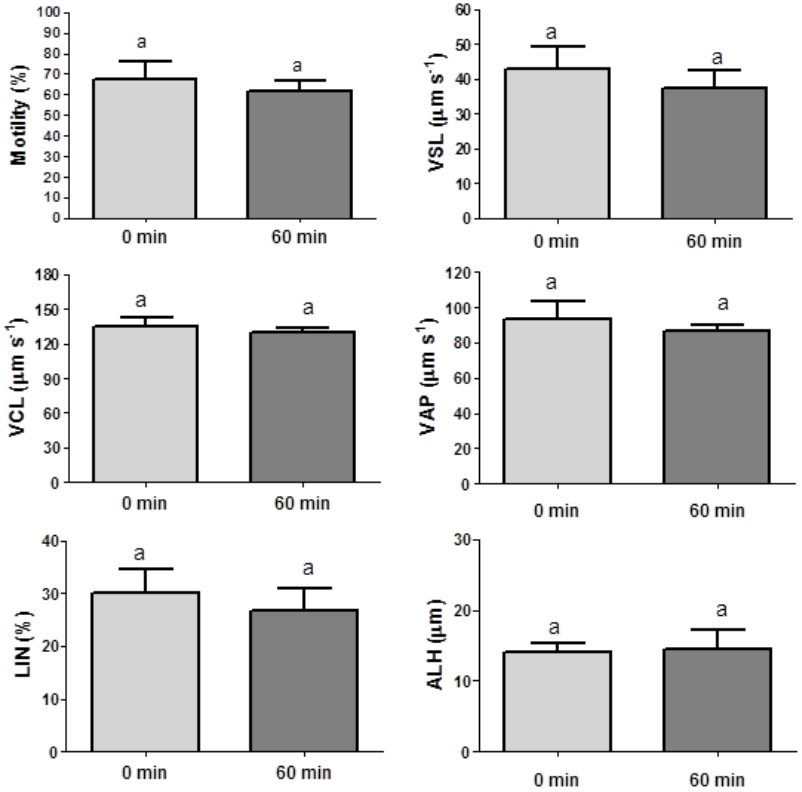


Brook trout

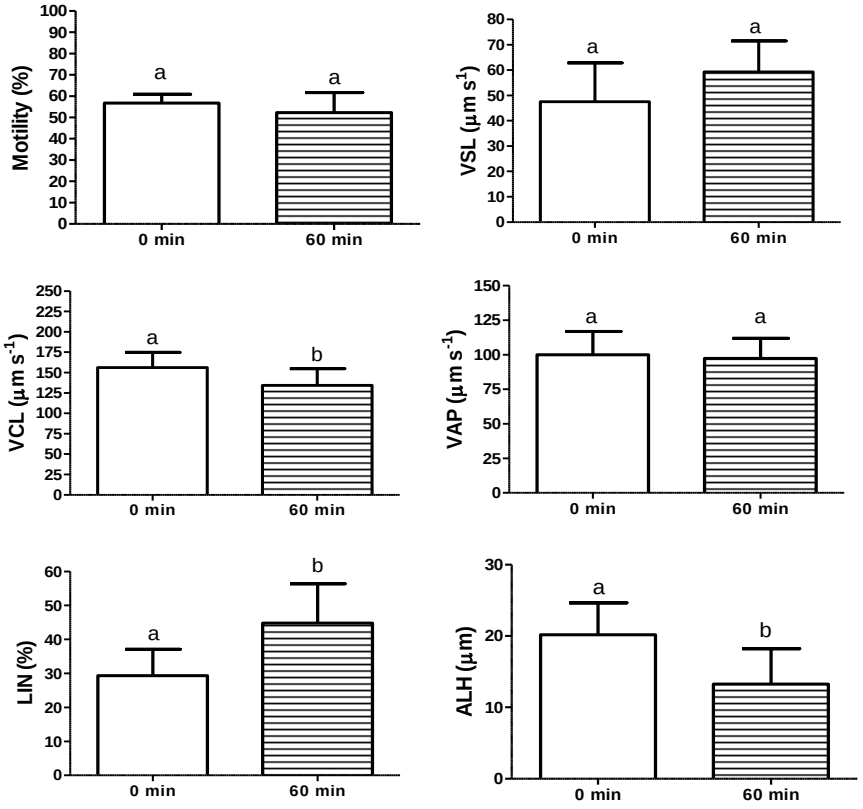


Effect of post-thaw sperm storage on motility parameters

Brown trout



Brook trout



Effect of post-thaw sperm storage on motility parameters

It had been assumed that thawed semen must be used immediately for fertilization within 30-second sperm storage after thawing significantly reduces the fertilization rate.
30/5s = 6 straws

60 (min) * 60 s = 3600 s/5s = 720 straws

Prolonged handling time of brook trout thawed semen could lead to better organization of hatchery work because the thawing procedure of several sperm samples for fertilization trials is time consuming.

Aquaculture 408–409 (2013) 30–33

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Technical Note

The use of concentrated extenders to improve the efficacy of cryopreservation in whitefish spermatozoa

A. Ciereszko [a,*], G.J. Dietrich [a], J. Nynca [a], E. Liszewska [a], H. Karol [a], S. Dobosz [b]

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Aquaculture 433 (2014) 62–65

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effect of cryopreservation on sperm motility parameters and fertilizing ability of brown trout semen

J. Nynca [a,*], G.J. Dietrich [a], S. Dobosz [b], J. Grudniewska [b], A. Ciereszko [a]

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Aquaculture 420–421 (2014) 275–281

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Cryopreservation of rainbow trout semen using a glucose-methanol extender

A. Ciereszko [a,*], G.J. Dietrich [a], J. Nynca [a], S. Dobosz [b], T. Zalewski [b]

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Aquaculture 435 (2015) 146–151

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Efficient method for cryopreservation of European huchen (*Hucho hucho* L.) and grayling (*Thymallus thymallus* L.) semen

Joanna Nynca [a,*], Grzegorz J. Dietrich [a], Joanna Grudniewska [b], Stefan Dobosz [b], Ewa Liszewska [a], Maksymilian Krzyś [c], Rafał Różyński [b], Andrzej Ciereszko [a]

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Aquaculture 434 (2014) 27–32

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Application of glucose–methanol extender to cryopreservation of semen of sex-reversed females rainbow trout results in high post-thaw sperm motility and fertilizing ability

G.J. Dietrich [a,*], J. Nynca [a], S. Dobosz [b], T. Zalewski [b], A. Ciereszko [a]

[a] Department of Gametes and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland
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Theriogenology 83 (2015) 253–256

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of postthaw storage time and sperm-to-egg ratio on fertility of cryopreserved brook trout sperm

J. Nynca [a,*], G.J. Dietrich [a], S. Dobosz [b], T. Zalewski [b], A. Ciereszko [a]

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Implementation

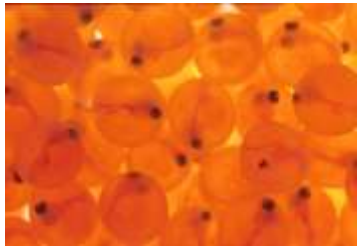
CryoSperm Bank

Cryopreserved sperm

Whitefish | Arctic charr | Brook trout



Fertilization



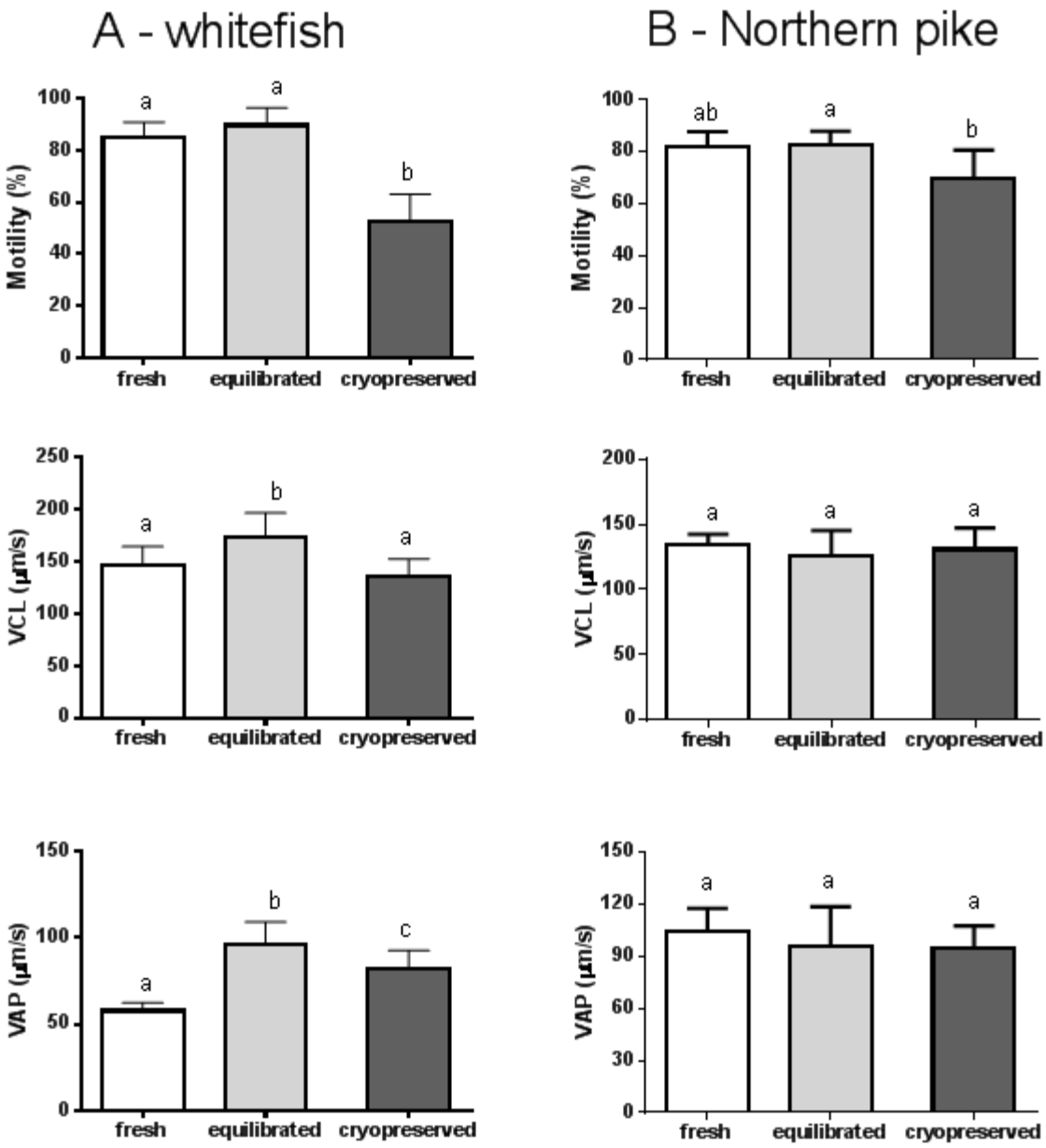
Gynogenetic	Hybrids	Interspecific hybrids
Lines of salmonid fish	„sparctic”	for ex. rainbow trout

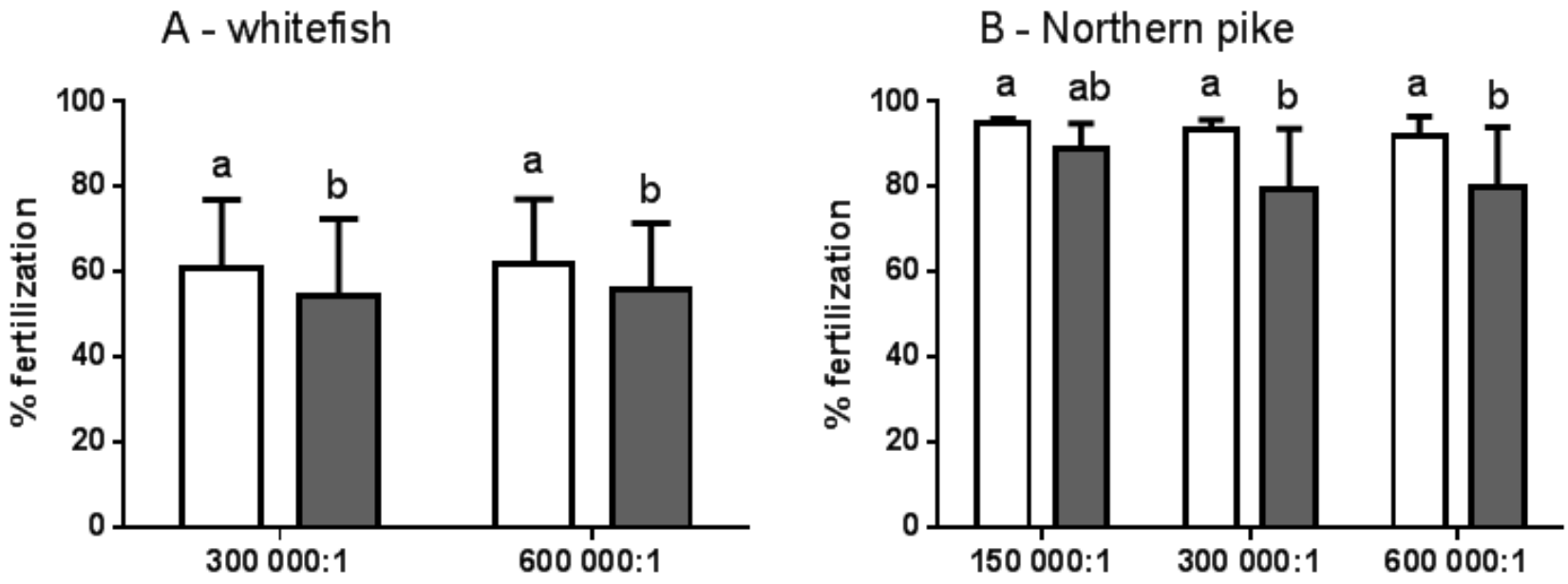
Further experiments

Different species

Improvement of technology

- **Addition of antioxidants (cysteamine, glutathione, etc., antioxidative enzymes)**
- **Different sugars (sucrose, trehalose, etc.)**
- **Anti-freeze proteins**
- **Potassium ions**
- **Buffers**
- **Higher volume of straws**
- **Higher sperm concentrations in straws**


A - whitefish
B - Northern pike
Motility (%)
VCL (µm/s)
VAP (µm/s)
fresh
equilibrated
cryopreserved
a
ab
b
c


A - whitefish
% fertilization
100
80
60
40
20
0
a
b
a
b
300 000:1
600 000:1
B - Northern pike
% fertilization
100
80
60
40
20
0
a
ab
a
b
a
b
150 000:1
300 000:1
600 000:1

Preliminary results

16 October 2015

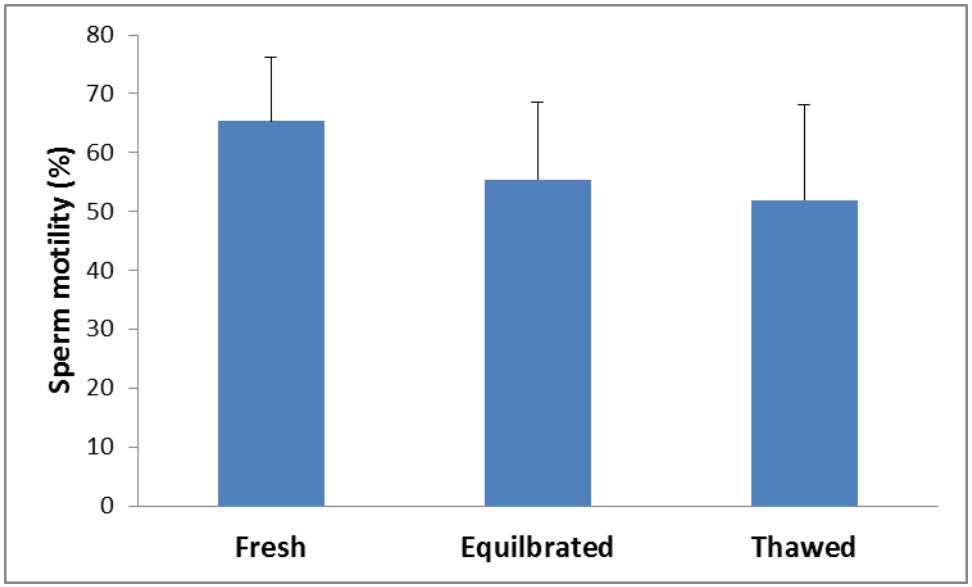
Cryopreservation of brown trout, cherry salmon and white-spotted char semen

Andrzej Ciereszko
Joanna Nynca
Mariola Dietrich
Konrad Ocalewicz

Nanae Fresh-Water Station

Director Etsuro Yamaha

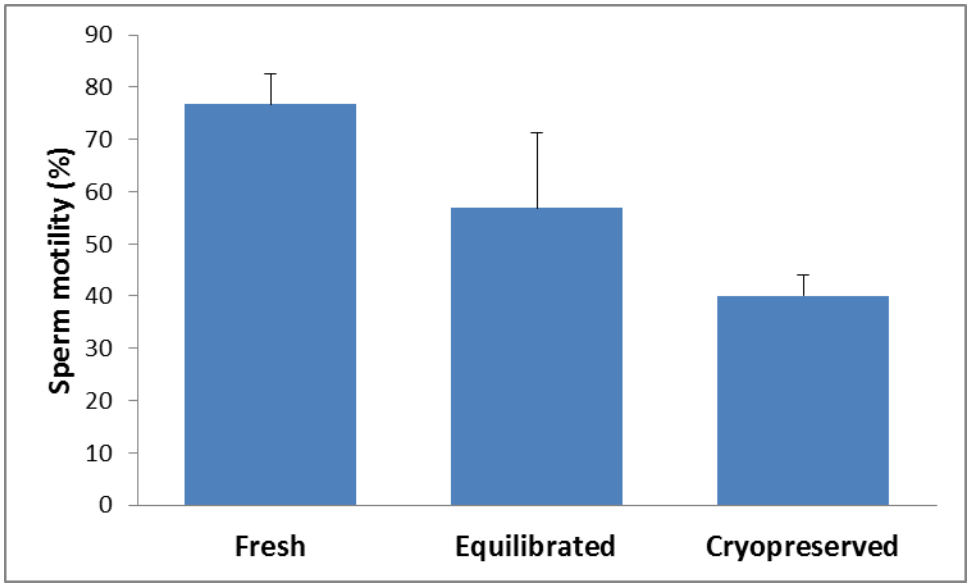
Brown trout



Male	Fresh	Equilbrated	Cryopreserved
1	53	47	35
2	70	60	60
3	80	70	70
4	57	37	35
5	67	63	60
Mean	**65.4**	**55.4**	**52**
SD	10.74	13.24	16.05

Cherry salmon *Oncorhynchus masou*

Cherry salmon

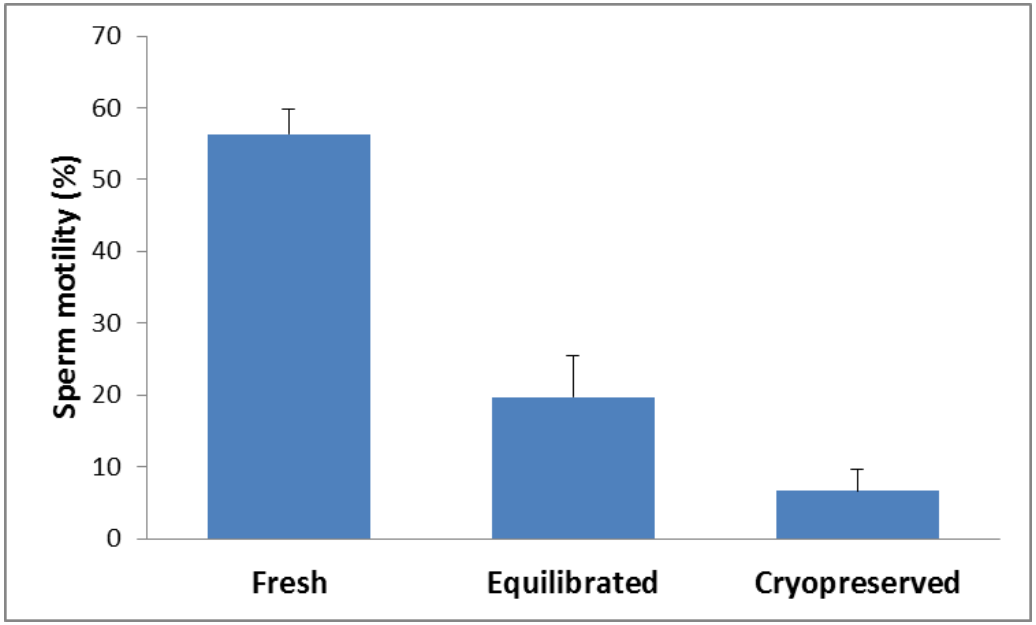


Male	Fresh	Equilibrated	Cryopreserved
1	66.7	47	37.5
2	80	67	42.5
3	80	77	45
4	77	43	35
5	80	50	40
Mean	**76.74**	**56.8**	**40**
SD	5.76	14.53	3.95

Very thick semen, observed agglutination of spermatoza after addition of extender to the fresh semen and in the thawed semen. Fish were at the end of spawning season.

White spotted char *Salvelinus leucomaenis*

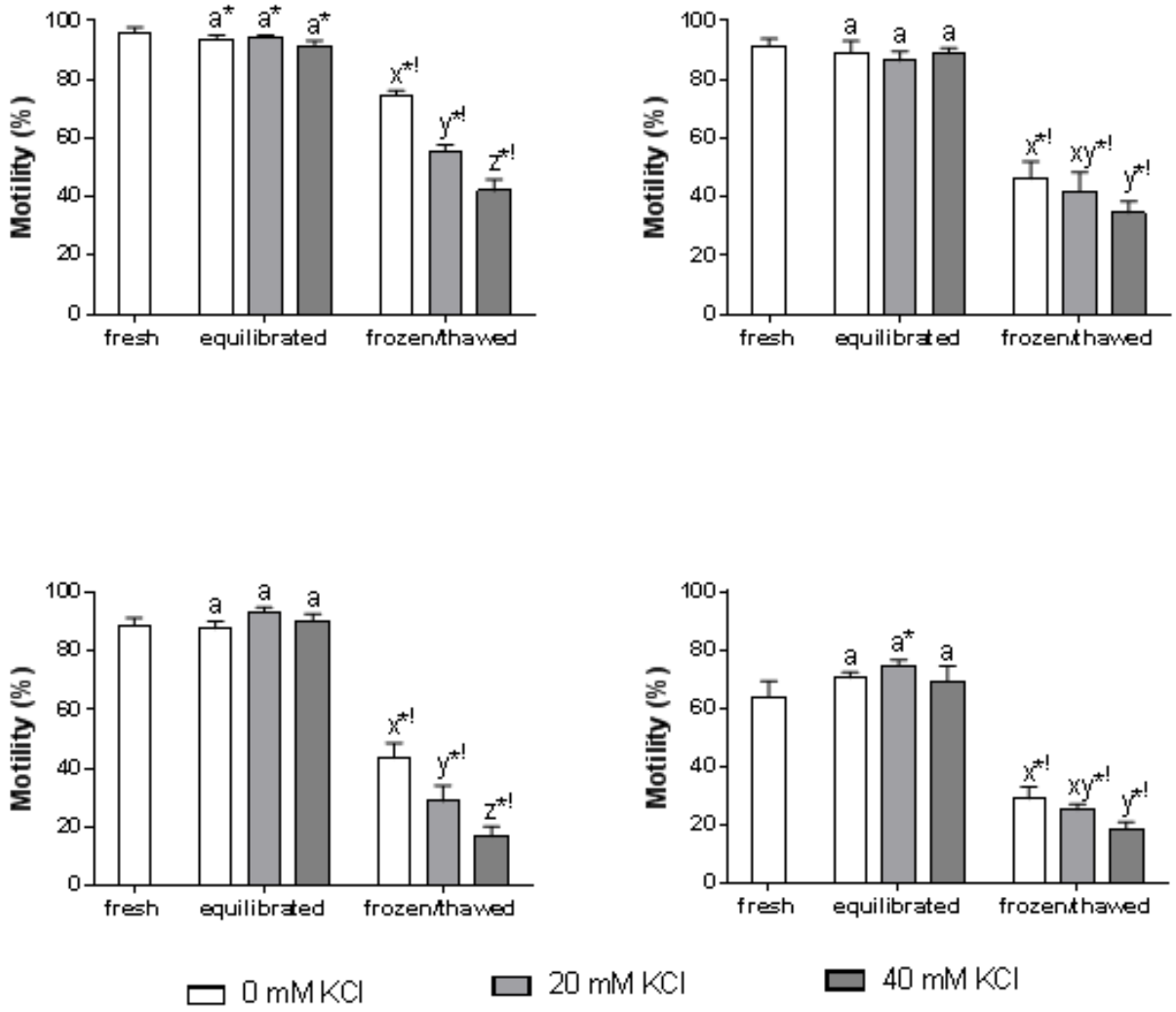
White spotted char



Male No.	Fresh	Equilibrated	Cryopreserved
1	56	23	5
2	60	23	10
3	53	13	5
Mean	**56.33**	**19.67**	**6.67**
SD	3.51	5.77	2.89

Agglutination of spermatoza after addition of extender and after thawing. Motility of the thawed sperm was short - 2-3 sec. Fish were sampled one day before experiment.

Effect of supplementation of glucose-methanol extender with potassium ions on sperm motility of salmonid fish



Replacement of glucose with trehalose or sucrose in GM extender

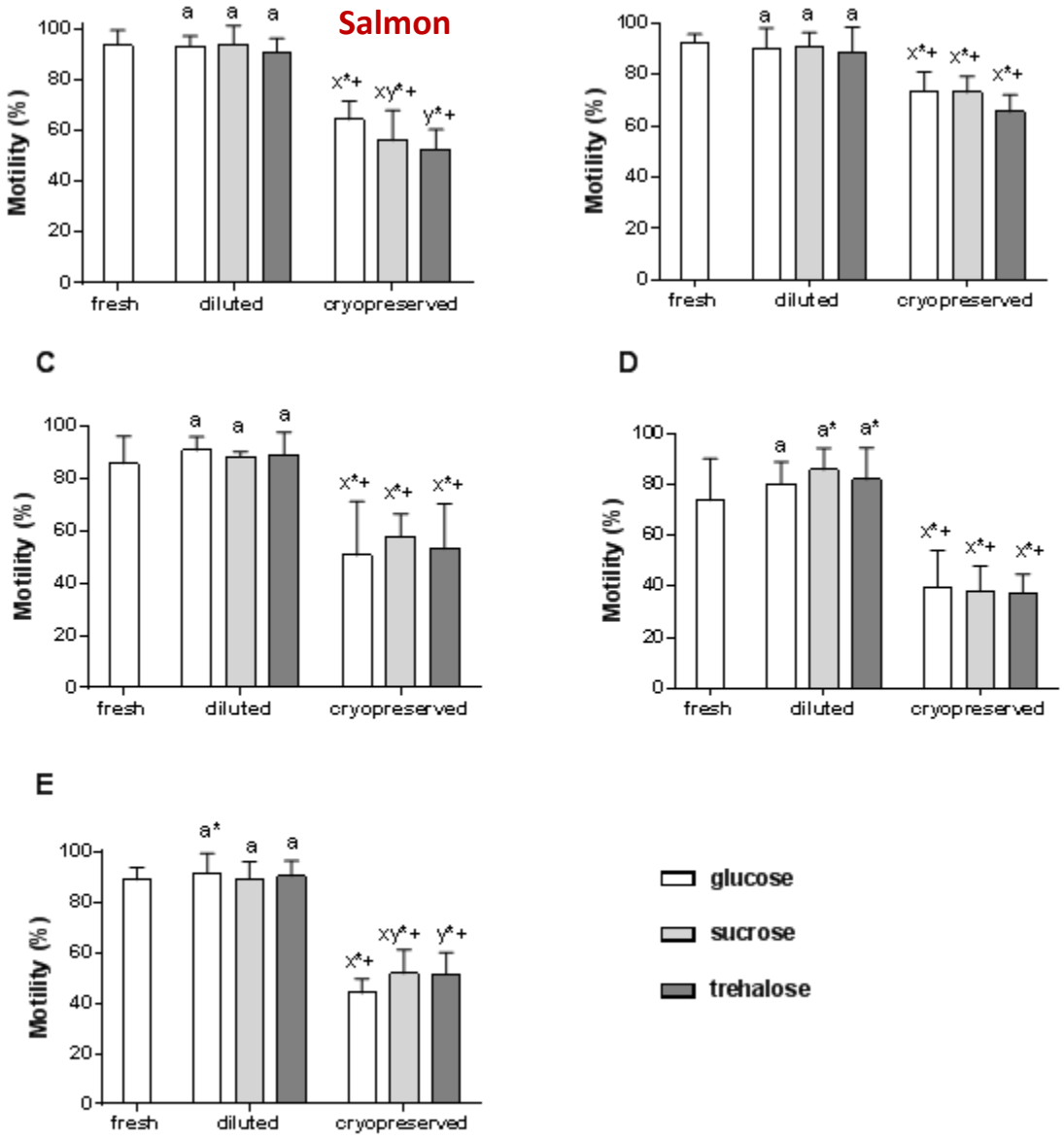


Fig. 1. Cryopreservation of A – salmon, B – brown trout, C – brook trout, D – sex-reversed female rainbow trout, E - whitefish semen with 9% methanol extender containing 0.18M glucose; 0.18M

Summary

GM extender seems to be well suited for cryopreservation of salmonid fish semen.

Species specific modification may be necessary. For example trehalose for whitefish.

The possibility of post-thaw semen storage for the prolonged time (at least 60 min) as well as the obtainment of high fertilization rate at low sperm-to-egg ratio can lead to the significant improvement in implementation of cryopreservation in hatchery practice.

Further studies should be focused on scaling up this efficient cryopreservation technique for application in hatchery conditions.

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