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Czech Republic

New possibilities of conservation of genetic resources

Vojtěch KAŠPAR, Roman FRANĚK, Martin PŠENIČKA

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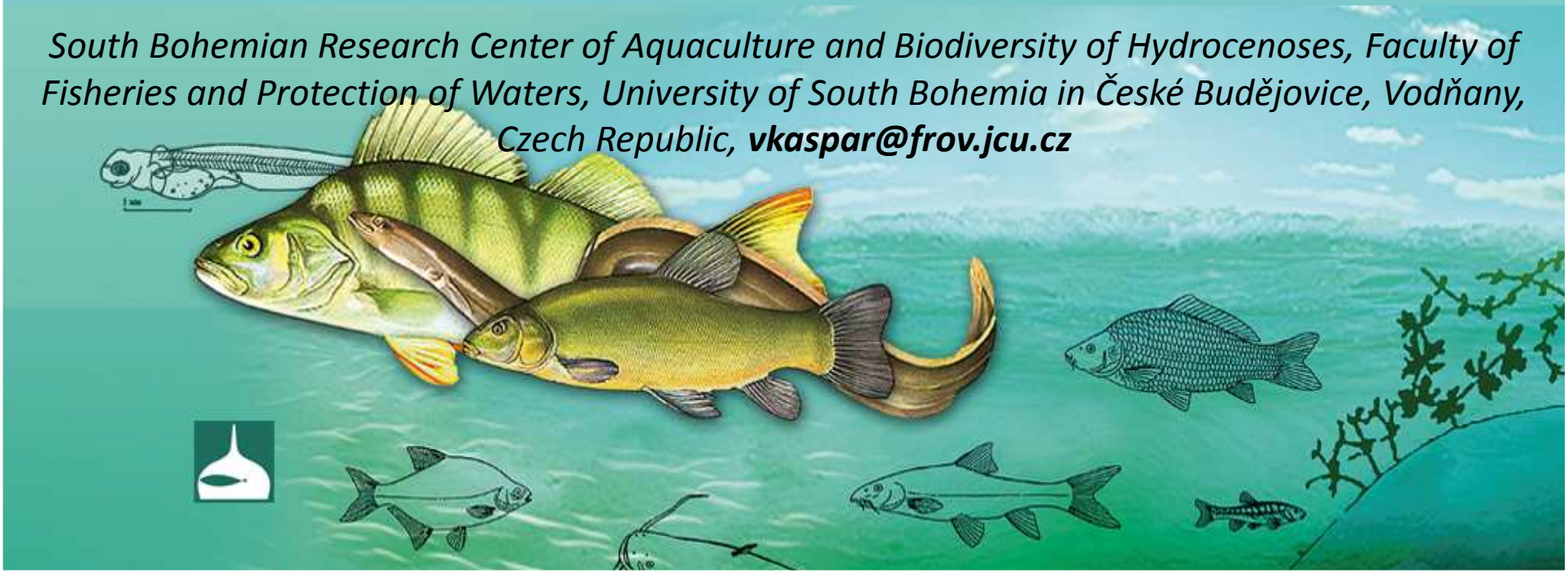

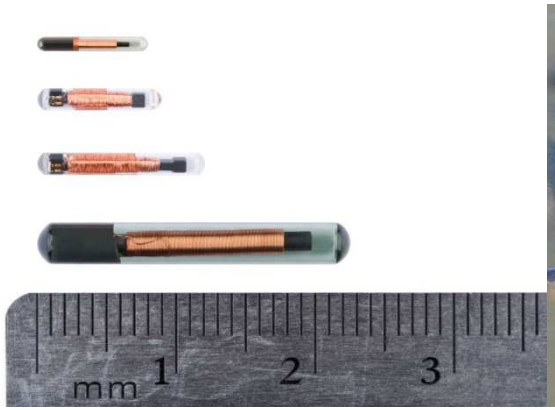

Urgent need for better management – meaning fully integrated use and conservation – of aquatic genetic resources for aquaculture:

in situ /in vivo, as free-living, wild and feral populations;

in situ /in vivo, as captive populations on-farm;

ex situ/in vivo as aquarium and research populations

ex situ/in vitro, as collections of cryopreserved sperm, DNA; (FAO, 2016).

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Conventional strategies of conservation of genetic resources of commercially important fish

Live gene banks – „in vivo“

- space requirements
- need to keep number of fish
- replicates needed
- broodstock management
- factorial design of reproduction
- risk of disseases

Conservation strategies – „in vitro“

- investment + operation costs
- limited use in freshwater species
- paternal gene pool conserved
- complete restoration of gene pool impossible
- long-term conservation

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WHY TO DO IT?

Table 3
Summary of the main cryobanks in Europe.

Name (country)	Purpose	Species	Type of frozen collection	Specificity	Costs coverage	Internet site or contact
Cryobank of the National Academy of Science (Ukraine)	Conservation Restoration Breeders	Wild fish: Carps, trouts, sturgeons, many rare species	Sperm	First fish sperm bank in Europe, sperm from extinct lines	Public funding	ekopeika@yahoo.com (Dr Evgeniy Kopeika)
Frozen Ark (UK)	Conservation	Wild fish from 112 UK species	Tissues, DNA, cells, blood	Preservation of biological know ledge	Public funding (Consortium)	www.frozenark.org
Cryo-Brehm (Germany)	Conservation Research	All wild animals, more than 20 fish species	Tissues, DNA, cells, cell lines, blood, sperm	Member of the Frozen Ark consortium Cell line provider for research	Public funding (Franhofer Inst.)	www.crvobrehm.de (phillip.ciba@emb.fraunhofer.de)
RIFCH Bank (Czech Republic)	Conservation Breeders	Farmed fish: 7 FW species, including 11 carp breeds	Sperm	Part of National Program for Conservation of FAGR (CZR)	Public funding	flajshans@frov.icu.cz (Prof Ing Martin Flajshans)
CryoAqua (France)	Conservation Breeders	Farmed resources: trout, oyster	Sperm	Both private storage/French National Cryobank (CRB-Anim)	Fees (private storage)/Public funding (conservation)	laboproduction35@evolution-xy.fr/www.crvobanque.org www.crb-anim.fr (clabbe@rennesinra.fr)
EZRC (Germany)	Research (EU)	Zebrafish	Sperm	Transgenic lines provider	Fee to get back the line	www.ezrc.kit.edu

Common carp sperm cryobanks also in NAIK-HAKI Szarvas (Hungary) , IIB PAN Golysz and IRS Olsztyn (Poland

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Alternative strategy of conservation of genetic resources of commercially important fish

• **Stem cells** - cells that can differentiate into other types of cells, and can also divide in self-renewal to produce more of the same type of stem cells.

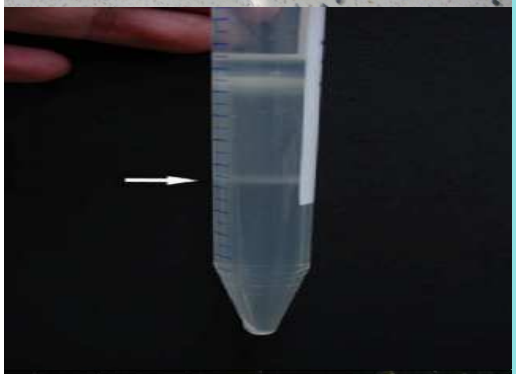
• **PGC –** cell gives rise to the gametes of an organism that reproduces sexually, originate in the primitive streak and migrate to the developing gonads, undergo meiosis followed by cellular differentiation into mature gametes, either eggs or sperm.

• **Spermatogonia** - is an undifferentiated male germ cell. Spermatogonia undergo spermatogenesis to form mature spermatozoa in the seminiferous tubules of the testis.

• **Oogonia** - a small diploid cell which upon maturation forms a primordial follicle resulting in formation of oocytes.


Juvenile specimens used for isolation of stem cells – spermatogonia or oogonia.

Gonadal tissue dissociated using enzyme solution.



Purification of dissociated gonadal tissue in density gradient – centrifugation.

Transplantation of purified stem cells to embryos of host – 10-12 days post hatching.

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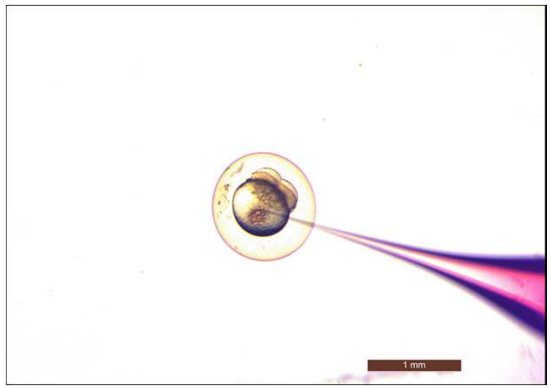
Transplantation strategy in conservation of genetic resources

Preparation of recipients

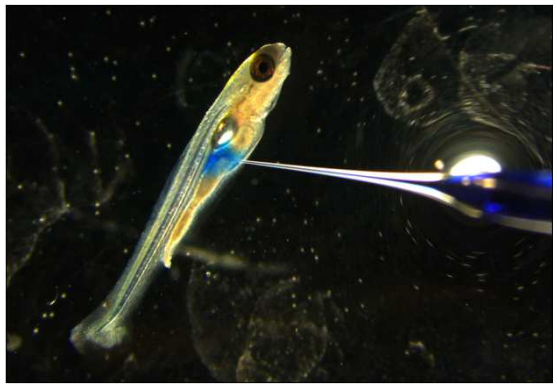
- Sterilization of recipients – gene knock down or triploidy induction



Cryopreserved cells

- Cryopreserved PGC, spermatogonia or oogonia

Tranplantation



PLOS ONE

RESEARCH ARTICLE

Cryopreservation and transplantation of common carp spermatogonia

Roman Franěk[1☯]*, Zoran Marinović[2☯], Jelena Lujić[2], Béla Urbányi[2], Michaela Fučíková[1], Vojtěch Kašpar[1], Martin Pšenička[1‡], Ákos Horváth[2‡]

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2 Department of Aquaculture, Szent István University, Gödöllő, Hungary

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
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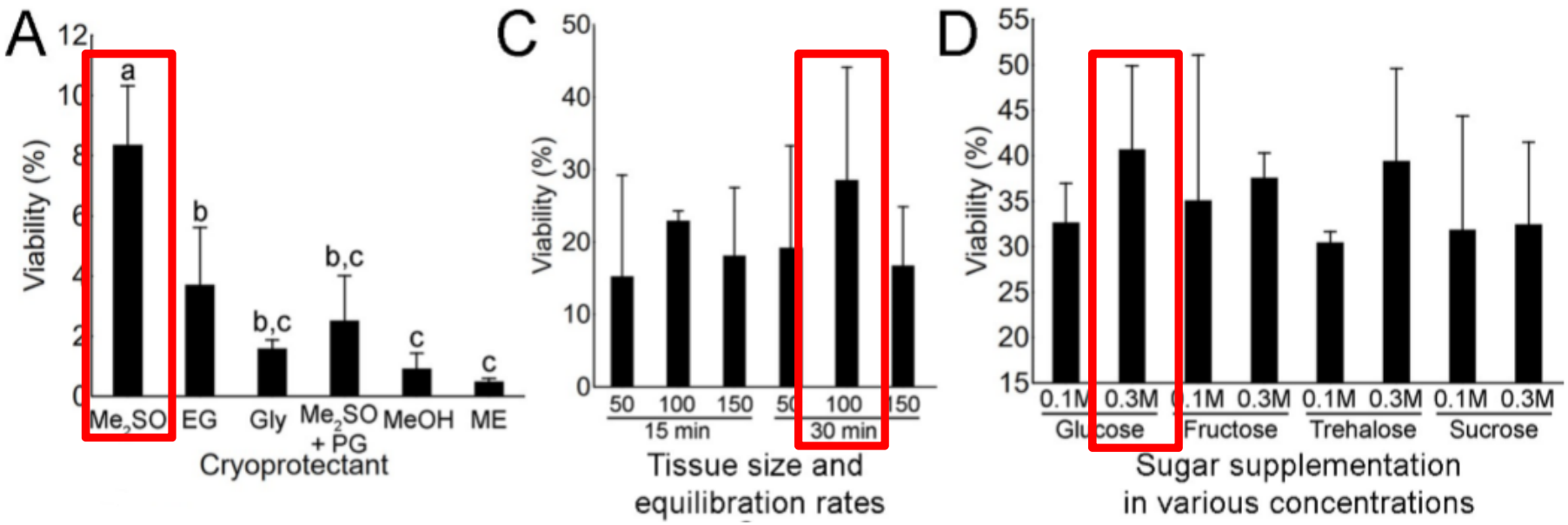
Control
40 / 40
17 / 23



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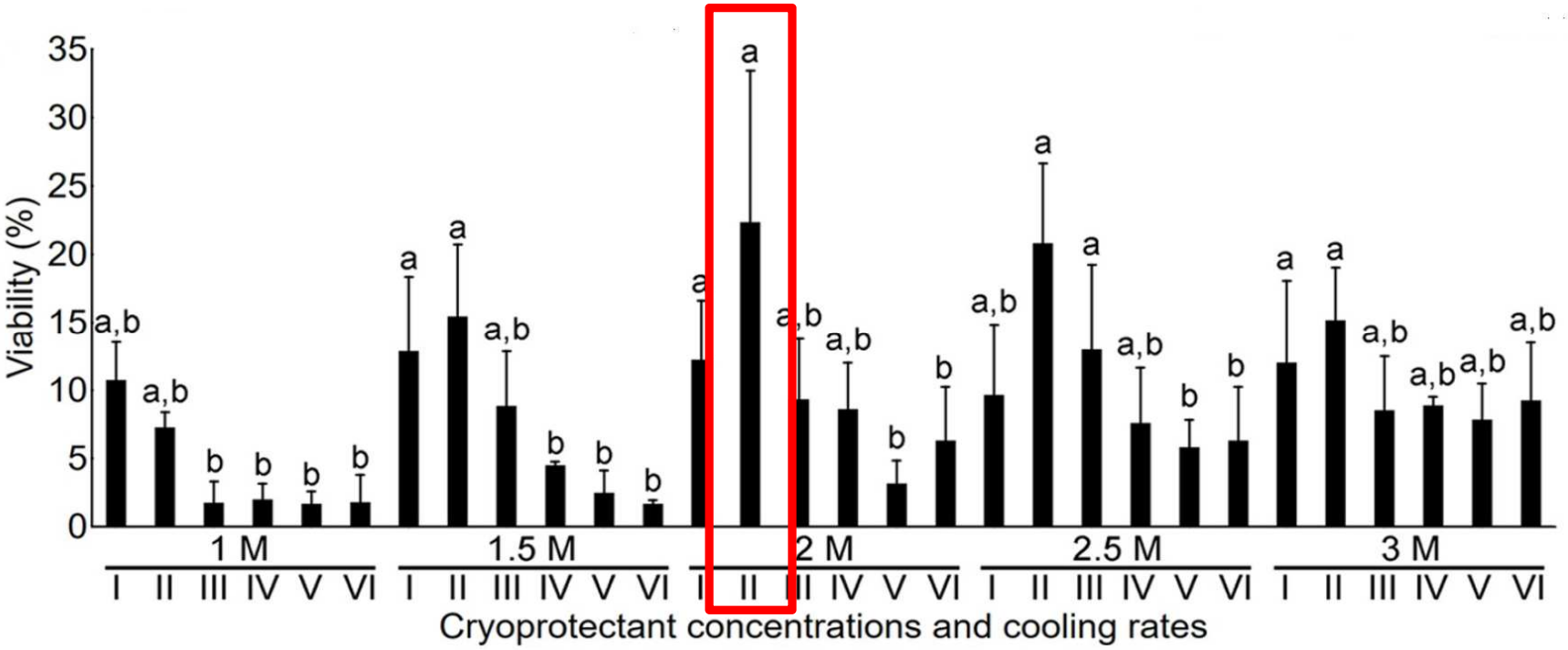

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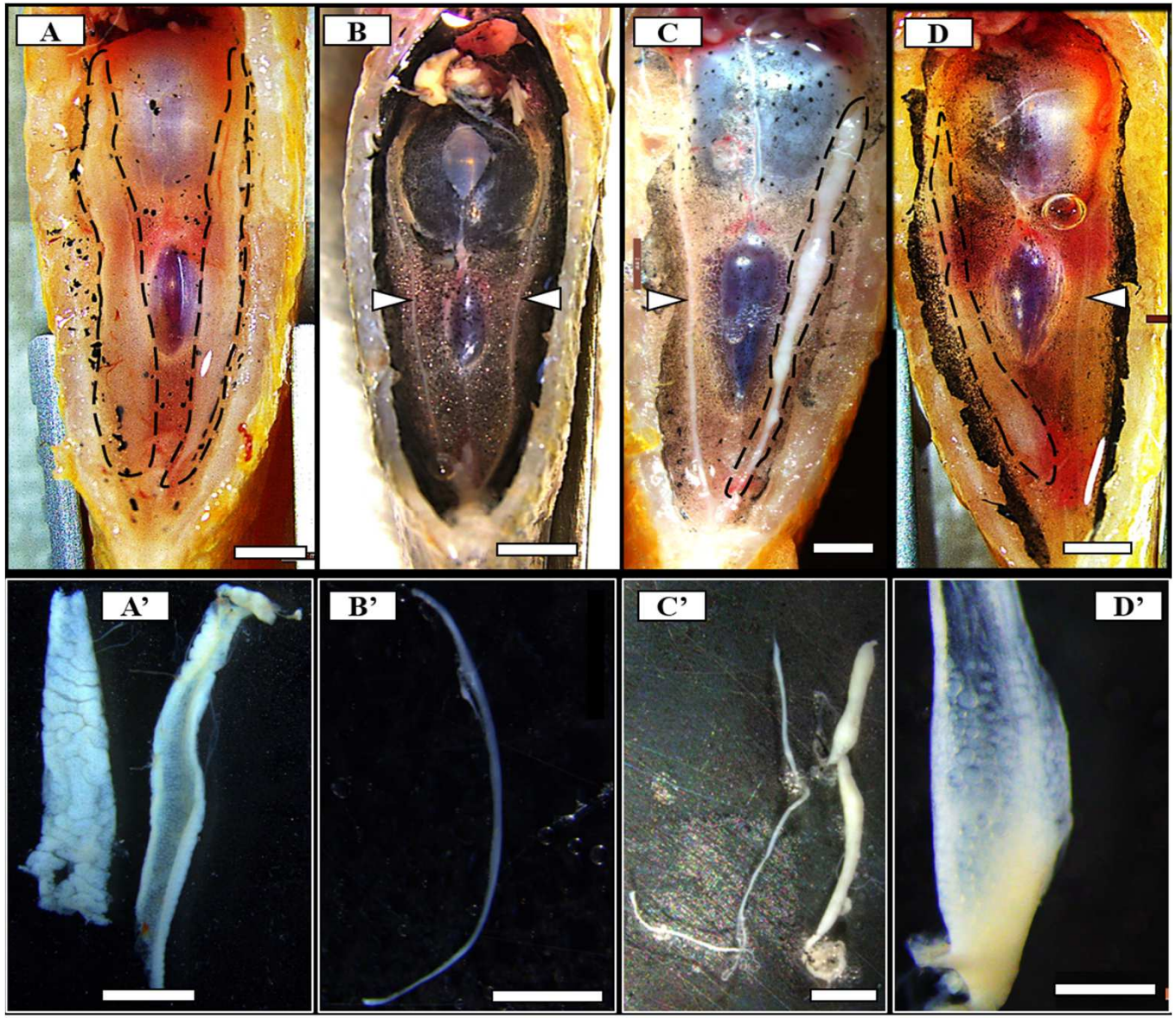


DEVELOPMENT OF GONADS – 3 months old specimens

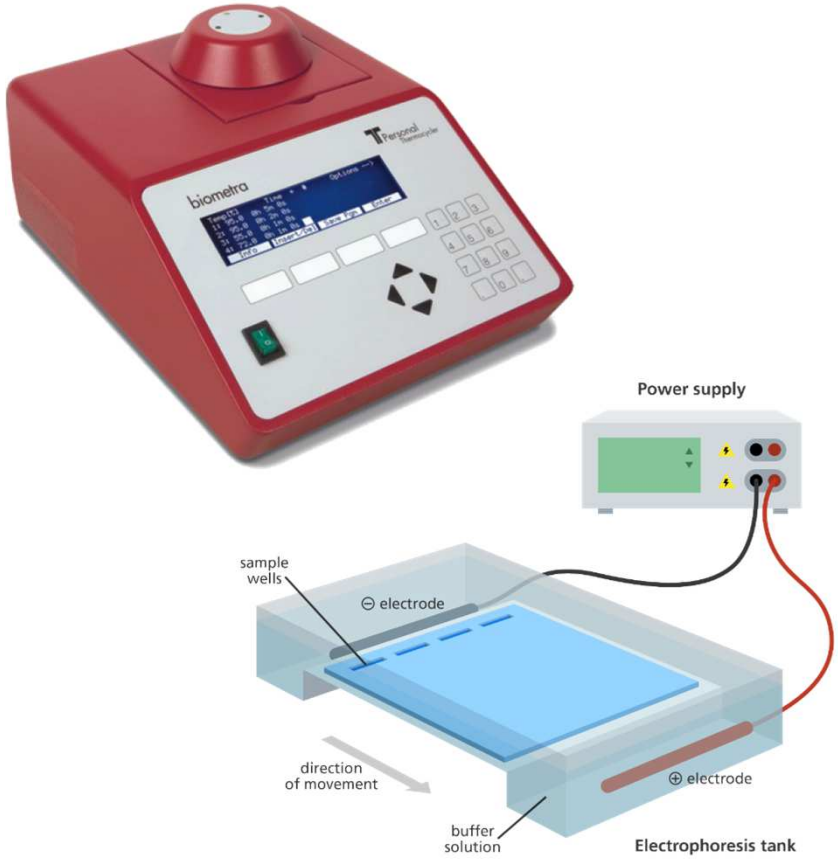
A) control treatment, boht sides developer

B) sterilized specimen, no gonads developeds

C) transplanted spermatogonia – developing testes

D) transplanted spermatogonia – developing ovary - Franěk et al (2019).

Treatment	Developed gonads	Testis / Ovary	Both gonads / one gonad	CARP	GOLDFISH
Control	40 / 40	17 / 23	40 / 0	0	40
Fresh cells transplanted	21 / 40	14 / 7	9 / 12	21	0
Cryopreserved cells transplanted	17 / 40	10 / 7	8 / 9	17	0
dnd MO treated	0 / 40	-	-	-	-



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journal homepage: www.elsevier.com/locate/cryo

Preservation of female genetic resources of common carp through oogonial stem cell manipulation

Roman Franěk[a], Tomáš Tichopád[a], Christoph Steinbach[a], Xuan Xie[a], Jelena Lujić[b], Zoran Marinović[b,*], Ákos Horváth[b], Vojtěch Kašpar[a], Martin Pšenička[a]

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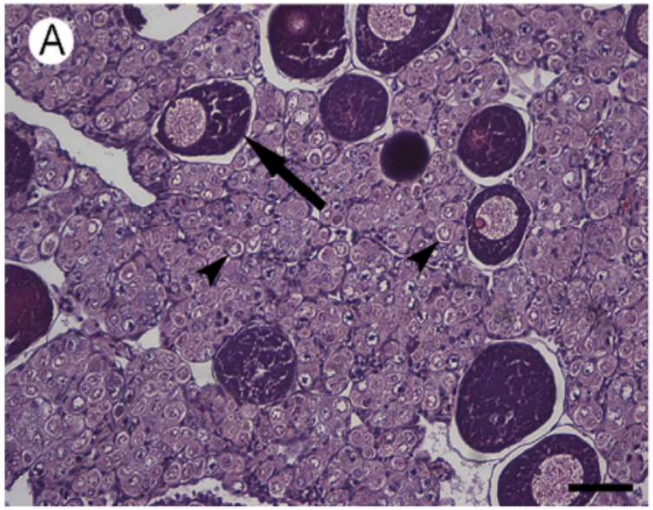
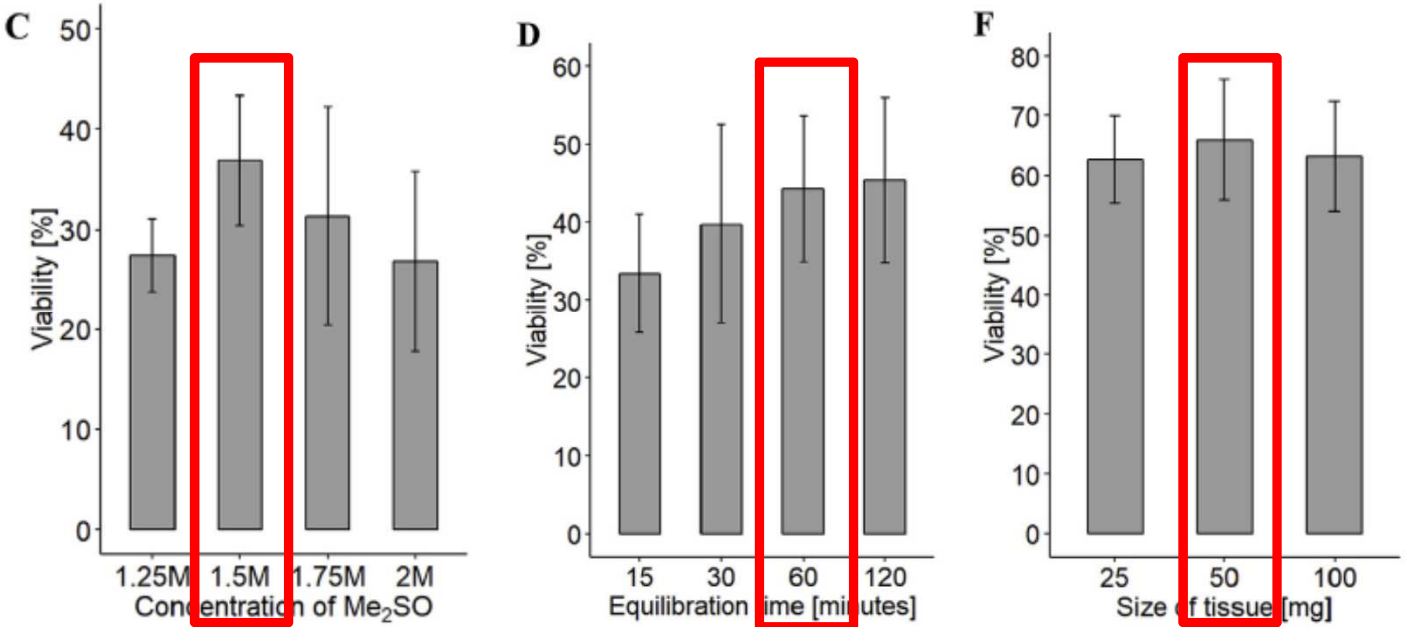
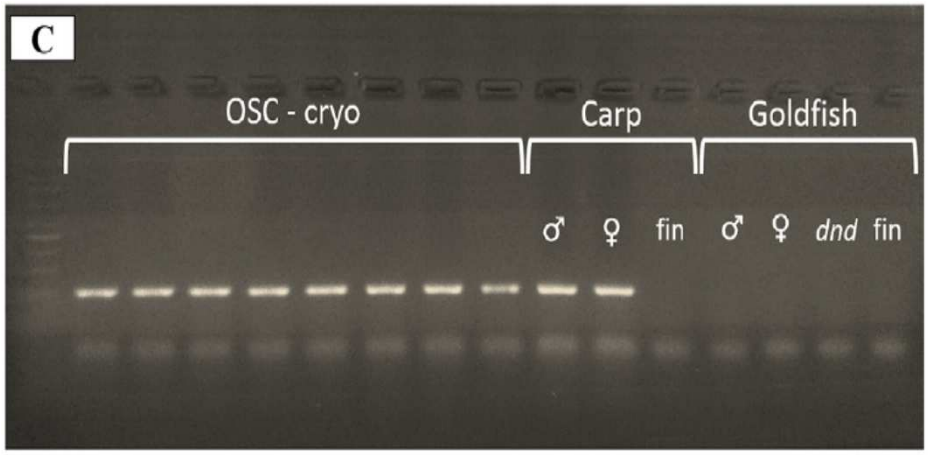
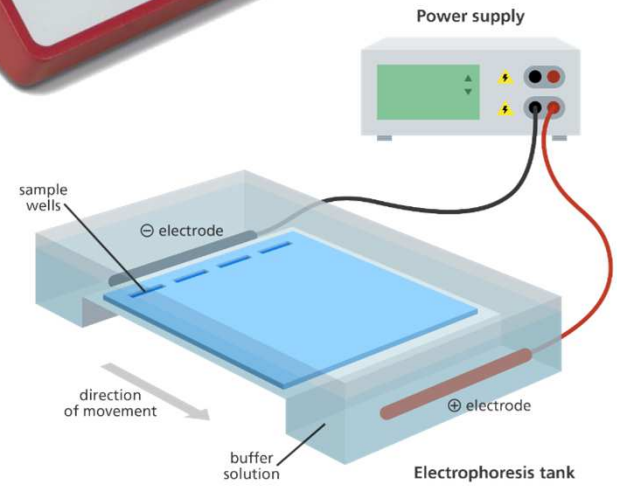
[b] Department of Aquaculture, Szent István University, Páter Károly u. 1, H-2100, Gödöllö, Hungary





Treatment	Transplanted	Survived (24h)	Survived (1 month)	Positive (1 month)	CARP positive (Carp dnd)
Control	80	80 / 100%	79 / 98,7%	0	0
Fresh cells transplanted	80	79 / 98,6%	72 / 90%	23 / 76,6%	18 / 60%
Cryopreserved cells transplanted	80	80 / 100%	69 / 86,2%	22 / 73,3%	19 / 63,3%
dnd MO treated	-	80 / 100%	78 / 97,5%	0	0





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- **cryopreservation of spermatogonia from developing gonad is well established!**
- **cryopreservation of oogonia from developing ovary is established!**
- **transplantation performed and transplanted cells develloped into gonadal tissue of species of origin**
- **sperm of common carp stripped from goldfish in 2018**
- **August 2019 – goldfish stripping, 2 groups of fish from transplantation of double haploid specimens – spermatogonia and oogonia transplantation**
- **August 2019 – goldfish stripping resulted in obtaining functional sperm cells and eggs from 2 females, confirmation of origin i progress**

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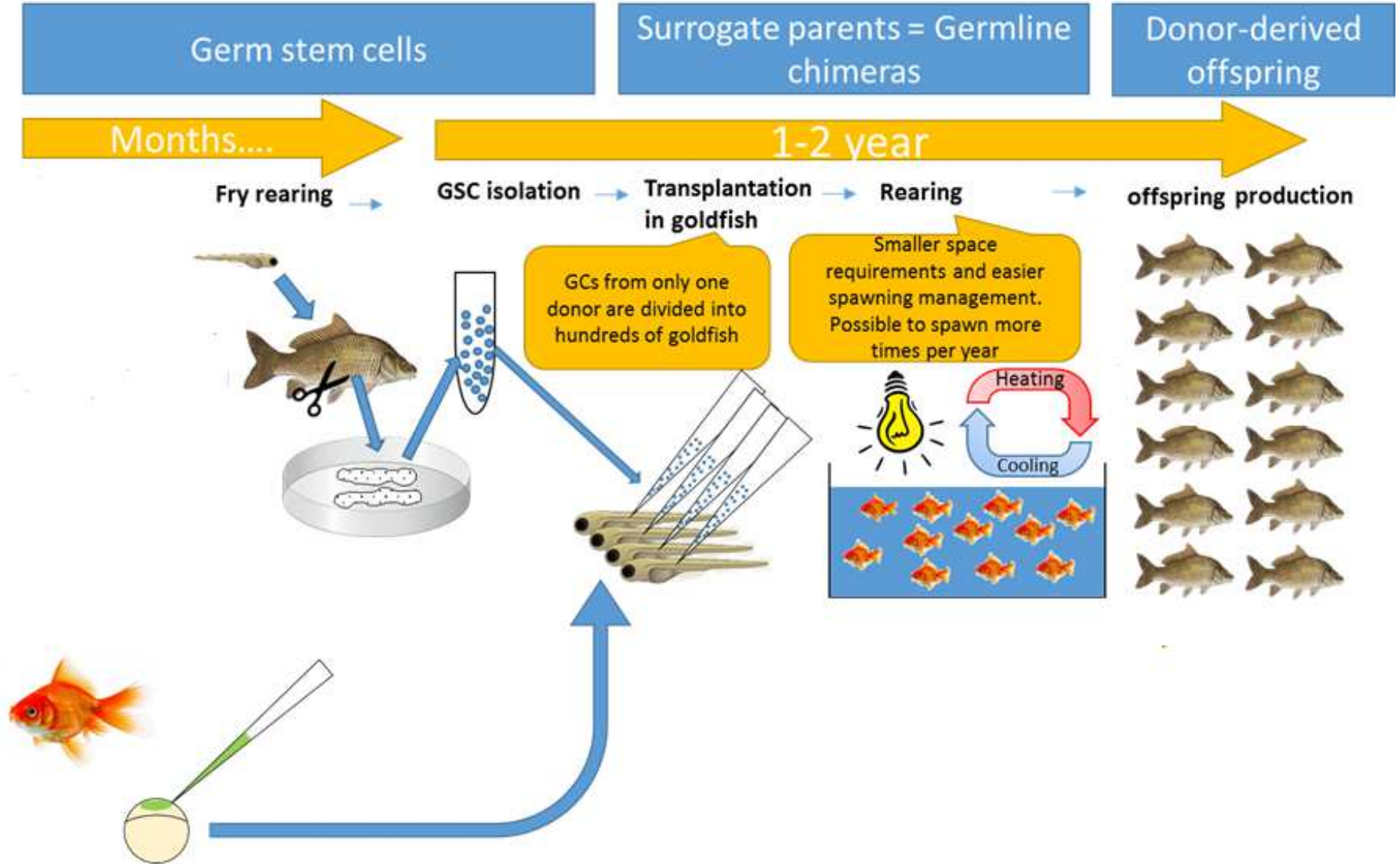

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RYBÁŘSTVÍ NOVÉ HRADY
PETR UVZDAR
s. r. o.

MINISTRY OF AGRICULTURE
OF THE CZECH REPUBLIC

QK1910428 In vitro conservation of genetic resources of common carp and creation of isogenic lines using transplantation of germ cells (2019-2023, responsible leader: Vojtěch Kašpar, Ph.D.)

- to implement alternative technology for conservation of genetic resources based on cryoconservation of male or female stem cells and conservation of genetic resources in vitro implementing system of surrogate production
- to set-up isolation and cryoconservation of germ stem stem cells, sterilization of common carp and goldfish, transplantation of germ stem cells of carp into body cavity of juvenile specimens of goldfish to obtain functional gametes of donor – common carp
- to enable effective creation of isogenic lines and establish new principles of in vitro conservation of genetic resources


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