Book of abstracts

8th International Workshop on the Biology of Fish Gametes

20-23 September 2022
Gdańsk
POLAND

8th International Workshop on the Biology of Fish Gametes

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8th International Workshop on the Biology of Fish Gametes

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Prefeace

The 8th International Workshop on the Biology of Fish Gametes is a continuation of previous excellent meetings of the scientific community having interest in gametes and reproduction-related technologies of aquatic organisms. The first Workshop was held in Vodňany (Czech Republic) back in 2007 and has since been followed up every two years with wonderful and inspiring events in Valencia (Spain), Budapest (Hungary), Faro (Portugal), Ancona (Italy) and Vodňany (10th anniversary event). The last event took place in Rennes (France) in 2019 where its organization was passed on to the Polish team of Institute of Animal Reproduction and Food Research of Polish Academy of Sciences. Initially, the meeting was planned to be organized in 2021, but it was later postponed due to the unprecedented global and tragic pandemic of COVID-19 whose long shadow is still being felt right now, together with another tragic event – an invasion of Ukraine by Russia, affecting the overall functioning of Central and Eastern Europe. However, after many reconsiderations and consultations with the International Scientific Committee, the Organizing Committee has decided to proceed and to organize the 8th edition of this meeting in September 2022 in a traditional fashion – in-person. We believe that face-to-face interactions at this particular conference are vital for integrating our scientific community which catalyzes the generation of new ideas, networks, and collaborations. Most importantly, after the difficult period of remote meetings we are looking forward to seeing our friends and colleagues as well as to meet new enthusiasts of fish gametes biology.

Having in mind the surrounding circumstances we were astonished by the great response of the scientific community to the announcements on the organization of this meeting and are deeply satisfied by receiving of 91 abstracts from 14 countries. Out of these, 40 abstracts were selected for oral presentations which are planned to be presented during 6 scientific session, including biotechnology and biotechniques, gametogenesis, gamete quality, gamete biology, fertilization and development, and gamete storage and cryopreservation. We are deeply honored to include in the program of this conference contributions of excellent, highly devoted and passionate scientists from the field. And we are happy that this event will be enriched by a plenary talk from Katsutoshi Arai from Hokkaido University, who has been involved in Fish Gametes workshops from the very beginning, as well as Ibon Cancio from University of the Basque Country (Spain) and Audrey Laurent from INRAE LPGP (Rennes, France) who kindly agreed to take part in building our exciting program. We are sure these talks will become inspiring for all of the members of our unique community.

Our ambition is to make your time in Poland fruitful, inspiring and pleasant.

So, welcome to Poland! Let's have some science-based fun together.

Andrzej Ciereszko

Daniel Żarski

8th International Workshop on the Biology of Fish Gametes

Meet the Honorary Speakers

Prof. Katsutoshi Arai

Plenary Lecturer

About me

My research life began in studying salmonid hybrids on 1970's. I performed cross-breeding using as many salmonid species as possible and then classified the resultant progeny into viable and inviable hybrids. Then, I analyzed enzyme genotypes and chromosomes in these hybrid embryos to understand genetic mechanisms underlying survival potential and developmental capacity. In 1980's, I combined hybrid studies and chromosome manipulation techniques and showed that artificial allotriploidization drastically increased survival potential of inviable hybrids to produce aquaculture breed.

From 1990's to present, chromosome manipulation techniques were studied for production of sterile triploids with outperformed growth in abalone as well as all-female population of flatfish. Production of gynogentic, androgenetic or polyploid progeny also contributed to estimate sex determination in flatfish, pufferfish, sturgeon and zebrafish.

In 21th century, research interest was shifted to natural polyploid and asexual fishes. Using natural fertile gametes of natural tetraploid dojo loach (Cobitidae), various kinds of auto- and allopolyploids were produced to analyze their reproductive characteristics. Mechanisms responsible for atypical reproduction such as unreduced gamete formation and natural cloning by gynogenesis have been investigated using natural clone dojo loach from the eastern part of Hokkaido, Japan by various experimental, genetic and cytogenetic approaches.

Prof. Ibon Cancio

Keynote Speaker

About me

I am a Professor in Cell Biology in the University of the Basque Country. I did my MSc at the University of Wales (UK) and European PhD at the University of the Basque Country, with research stays in Universities of Amsterdam, Heidelberg and Leuven. I coordinated the MSc degree Environmental Contamination & Toxicology for 10 years at the University of the Basque Country.

I am currently a researcher of the consolidated research group Cell Biology & Environmental Toxicology in the Plentzia Marine Station of the University of the Basque Country, participating in over 55 regional, national and European research projects. My research focuses in the cell/molecular biology of fish sex differentiation and molecular biomarkers of endocrine disruption, mainly xenoestrogenicity. So in principle I am a cell biologist with a foot in ecotoxicology and another one in the study of fundamental processes associated to development, reproduction and aquaculture.

Personally I like Rock and Roll and enjoy a goof rugby match, especially if Wales is playing. And when comes to reproduction, but this time mammalian, I am a father of two children.

Dr. Audrey Laurent

Keynote Speaker

About me

I did my PhD and first post doc working on very early development in xenopus and mouse. I mainly studied a family of transcription factors (Pbx and Meis/Prep), which were famous as hox co-factors, doing expression profiles and gene invalidation to show that these proteins actually have unexpected roles in early development, such as the control of chromatin compaction or the maintenance of genome integrity. I also worked on mouse ES cells to develop *in vitro* differentiation assays and ChIP experiments.

I came back to France for a second post-doc where I discovered 'omic' approaches. Combining RNAseq and ChIPseq I studied the epigenetic reprogramming of human breast cancer cells upon the modulation of methylation.

Since 4 years I am working at the Laboratory of Fish Physiology and Genomics of INRAE in Rennes, France, where I am developing research on fish gametes epigenetics.

CONFERENCE SCHEDULE

TUESDAY, 20 SEPTEMBER 2022

9.00-9.20 Welcome and opening ceremonies

PLENARY LECTURE

9.20-10.00 **Arai K.**: Chromosomally manipulated and naturally occurred polyploid and unisexual fish: lessons from the dojo loach (*Misgurnus anguillicaudatus*)……**pp. 15 [O1]**

10.00-10.40 Coffee break

SESSION 1:
BIOTECHNOLOGY AND BIOTECHNIQUES
Chairman: Martin Pšenička & Elvira Fatsini

10.40-11.00 **Nayak R.**, Franěk R., Šindelka R., Pšenička M.: Enhancement of zebrafish gamete production in a big body sized giant danio by germ cell transplantation……**pp. 17 [O2]**

11.00-11.20 Goupil A.-S., Kica S., Terrenne T., Goardon L., Depince A., Labesse C., Lareyre J.-J., **Labbé C.**: Cryopreservation of rainbow trout germinal stem cells: application in aquaculture to restore both spermatozoa and oocytes from valuable genotypes……**pp. 18 [O3]**

11.20-11.40 Bhat I.A., **Dubiel M.**, Jónsson Z.O., Rodriguez E.: Molecular biology tools for sterile salmon production……**pp. 19 [O4]**

11.40-12.00 **Fujimoto T.**, Kawamura Y., Nishimura T., Arai K.: Tetraploidization recovers fertility in sterile inter-group hybrid males of dojo loach……**pp. 20 [O5]**

12.00-12.20 **Ocalewicz K.**: Quality of rainbow trout eggs and efficiency of androgenesis and gynogenesis……**pp. 21 [O6]**

12.20-12.40 **Kitanović N.**, Marinović Z., Pataki B., Mészáros G., Saho S., Urbányi B., Horváth Á.: Optimization of culture media for *in vitro* maturation of common carp ovarian follicles……**pp. 22 [O7]**

12.40-13.40 Lunch break

SESSION 2:
GAMETOGENESIS
Chairman: Juan Asturiano & Joanna Nynca

13.40-14.00 **Oliveira C.C.V.**, Fatsini E., Santos T., Hubbard P., Velez Z., Cabrita E.: Kisspeptin treatment induced reproductive traits during puberty and altered chemical communication in Senegalese sole……**pp. 24 [O8]**

14.00-14.20 **Giommi C.**, Zanardini M., Maradonna F., Habibi H.R., Carnevali O.: Insights on reproductive gender specific toxicity induced by glyphosate chronic exposure in *Danio rerio* adults……**pp. 25 [O9]**

14.20-14.40 Roza de Abreu M., Janati Idrissi S., de Mello F., Guyomar C., Nguyen T., Pšenička M., Herpin A., Thermes V., Seitz H., **Bobe J.**: Hippo pathway-mediated regulation of micropyle formation by micro RNA 202 (miR-202) in the fish oocyte……...**pp. 26 [O10]**

14.40-15.00 **Nobrega R.H.**, Doretto L.B., Thomas M., Chenais N., Lareyre J.-J.: FSH regulates the proliferation of embryonic-like germ stem cells in adult zebrafish testes……**pp. 27 [O11]**

15.00-15.40 Coffee break

SESSION 3:
GAMETES QUALITY
Chairman: Julien Bobe & Taina Rocha De Almeida

15.40-16.00 **Momin M.**, Memiş D.: Effect of *Moringa oleifera* supplemented diet on the sperm quality of male rainbow trout (*Oncorhynchus mykiss*)……**pp. 29 [O12]**

16.00-16.20 **Ljubobratović U.**, Kitanović N., Milla S., Marinović Z., Fazekas G., Stanivuk J., Żarski D., Horváth Á.: Predicting the egg quality based on the oocyte diameter and *in vitro* maturation in domesticated pikeperch (*Sander lucioperca)*…..**pp. 30 [O13]**

16.20-16.40 Valcarce D.G, Riesco M.F., Cuesta L., Martínez-Vázquez J.M., **Robles V.**: Unpredictable chronic stress alters behaviour, sperm quality and NMD pathway in zebrafish……...**pp. 31 [O14]**

16.40-17.00 **Félix F.**, Gallego V., Mendes A., Soares F., Vera L., Cabrita E., Oliveira C.C.V.: Species-specific melatonin patterns in fish seminal plasma……**pp. 32 [O15]**

WEDNESDAY, 21 SEPTEMBER 2022

PLENARY KEYNOTE

9.00-9.40 **Cancio I.**, Bir J., Nzioka A., Rojo-Bartolomé I., Ortiz-Zarragoitia M., Diaz de Cerio O.: It takes two to tango and if you are a fish oocyte you will also need enough ribosomes.....**pp. 34 [O16]**

SESSION 4:
FERTILIZATION AND DEVELOPMENT
Chairman: Oliana Carnevali & Marta Lombo

9.40-10.00 **Bernáth G.**, Láng Z.L., Várkonyi L., Fodor F., Nagy B., Bodnár A., Koltai T., Csókás E., Molnár J., Csorbai B., Csenki-Bakos Z., Ivánovics B., Szári Z., Urbányi B., Bokor Z.: The growth performance of pond reared larvae propagated using cryopreserved sperm in common carp (*Cyprinus carpio*)……**pp. 36 [O17]**

10.00-10.20 **Gazo I.**, Naraine R., Franěk R., Dietrich M.A., Flajshans M., Šindelka R., Pšenička M.: DNA damage response in sturgeon (*Acipenser ruthenus*) embryo: link between phenotype and transcriptome……**pp. 37 [O18]**

10.20-11.00 Coffee break

11.00-11.20 **Shah M.A.**, Saito T., Pšenička M.: Sturgeon (*Acipenser*) represents the evolutionary transition from the holoblastic to meroblastic cleavage pattern and unique gut development……**pp. 38 [O19]**

11.20-11.40 **Nagy B.**, Bokor Z., Csorbai B., Várkonyi L., Molnár J., Bartucz T., Láng Z.L., Ittzés Á., Ittzés I., Urbányi B., Bernáth G.: The investigation of the differences in artificial propagation and larvae development by four different goldfish types (*Carassius auratus*)……**pp. 39 [O20]**

11.40-12.00 **Chemello G.**, Cerrone G., Tavolazzi V., Donato F., Tiralongo F., Carnevali O., Gioacchini G.: Reproductive biology of the European sardine (*Sardina pilchardus*) in the Adriatic Sea, an integrated study evaluating ovary structural and morphological integrity within an entire reproductive cycle……**pp. 40 [O21]**

SESSION 5:
GAMETE BIOLOGY
Chairman: Elsa Cabrita & Luz Pérez

12.00-12.20 **Boryshpolets S.**, Dzyuba B., García-Salinas P., Gallego V., Sotnikov A., Asturiano J.F.: Sperm behaviour and motility in *Elasmobranch* species: the role of viscosity……**pp. 42 [O22]**

12.20-12.40 **Rusco G.**, Esposito S., Di Iorio M., Antenucci E., Roncarati A., Iaffaldano N.: The effectiveness of ovarian fluid on spermatozoa performances in Mediterranean brown trout wild population (Molise region - Italy)……**pp. 43 [O23]**

12.40-13.40 Lunch break

13.40-14.00 **Kholodnyy V.**, Dzyuba B., Cosson J., Boryshpolets S.: The kicker, the hugger and the catcher: ovarian fluid specifically determinates gametes encounter in rainbow trout, sterlet and common carp in line with their reproductive behaviour……**pp. 44 [O24]**

14.00-14.20 **Król J.**, Krejszeff S., Palińska-Żarska K., Żarski D.: Effect of light colors on spermiation and sperm kinetics parameters during out of season reproduction in pond-reared Eurasian perch (*Perca fluviatilis* L)……..**pp. 45 [O25]**

14.20-14.40 **Ferrão L.**, Blanes-García M., Pérez L., Asturiano J.F., Morini M.: First description of superoxidase dismutase multigene in European eel males: expression *in vivo* under different environmental conditions……**pp.46 [O26]**

14.40-15.00 **Kodzik N.**, Ciereszko A., Szczepkowski M., Karol H., Judycka S., Malinowska A., Świderska B., Dietrich M.A.: Proteomic analysis of Siberian sturgeon seminal plasma through shotgun and gel-based methods……..**pp. 47 [O27]**

15.00-15.40 Coffee break

15.40-16.00 Photo of Participants

16.00-17.00 POSTER SESSION

19.00-23.00 Student reception

THURSDAY, 22 SEPTEMBER 2022

PLENARY KEYNOTE

9.00-9.40 **Laurent A.**: Epigenetics in fish gametes: sensitivity to environmental clues and transgenerational acclimation……..**pp. 49 [O28]**

SESSION 6:
GAMETE STORAGE AND CRYOPRESERVATION (Part I)
Chairman: Ákos Horváth & Sylwia Judycka

9.40-10.00 **EL Kamouh M.,** Brionne A., Laurent A., Labbé C.: Cryopreservation affects cellular quality but not DNA methylation profile in rainbow trout spermatozoa……**pp. 51 [O29]**

10.00-10.20 Sotnikov A., Urbansky A., Alsved J., Boryshpolets S., Rodina M., **Dzyuba B.**: Sperm fractionation in fishes: density gradient centrifugation and microfluidic separation approaches for cryopreserved samples.....**pp. 52 [O30]**

10.20-11.00 Coffee break

11.00-11.20 **Cheng Y.**, Vechtova P., Samarin A.M., Samarin A.M., Brionne A., Zhang S., Linhartová Z., Xin M., Schumacher F., Sterba J., Labbé C., Linhart O.: Effect of short-term storage sperm in common carp (*Cyprinus carpio*) on sperm DNA methylation and epigenetic inheritance level in embryos……**pp. 53 [O31]**

11.20-11.40 **Blanes-García M.**, Marinović Z., Šćekić I., Lujić J., Ferrão L., Morini M., Urbányi B., Horváth Á., Asturiano J.F.: Cryopreservation of the testicular tissue and xenotransplantation of European eel *(Anguilla anguilla)* spermatogonia...**pp. 54 [O32]**

11.40-12.00 **García-Salinas P.**, Gallego V., Asturiano J.F.: Next steps on the application of techniques for the control of reproduction of chondrichthyan species.**pp. 55 [O33]**

12.00-12.20 **França T.S.**, González-López W.A., Sanchez M.P., Pérez L., Mañanós E.L., Felip A., Gómez A., Asturiano J.F.: Protocol optimization for sea bass sperm cryopreservation and assessment of post-thawing dilution to prolong sperm usefulness in aquaculture Mediterranean species……..**pp. 56 [O34]**

12.20-12.40 **Li N.**, Gavin-Plagne L., Francke S., Goardon L., Labbé C., Schmitt E.: Effect of an antibiotic-free medium on milt motility and fertility of rainbow trout semen after mid-term storage at 4°C……..**pp. 57 [O35]**

12.40-13.40 Lunch break

13.40-15.30 Free time

15.30-17.00 Shuttle to Malbork (departure from the conference venue)

17.30-23.00 Castle sightseeing and Gala Dinner (Malbork Castle)

FRIDAY, 23 SEPTEMBER 2022

SESSION 6:
GAMETE STORAGE AND CRYOPRESERVATION (Part II)
Chairman: Catherine Labbé & Victor Gallego

10.00-10.20 **Gao L.**, Shah M.A., Franěk R., Pšenička M.: Replacement of maternal germ plasm in sturgeon.....**pp. 59 [O36]**

10.20-10.40 **Samarin A.M.**, Schumacher F., Kleuser B., Waghmare S.G., Policar T., Linhart O., Samarin A.M.: Embryos produced from post-ovulatory aged oocytes in common carp (*Cyprinus carpio*) exhibit aberrant epigenetic modifications of global DNA hydroxymethylation……..**pp. 60 [O37]**

10.40-11.20 Coffee break

11.20-11.40 **Waghmare S.G.**, Samarin A.M., Samarin A.M., Danielsen M., Møller H.S., Policar T., Linhart O., Dalsgaard T.K.: Histone modifications during oocyte ageing in common carp (*Cyprinus carpio*)……**pp. 61 [O38]**

11.40-12.00 **Horváth Á.**, Pataki B., Mészáros G., Staszny Á., Marinović Z., Kitanović N., Urbányi B.: Investigation of inherited cryoresistance in zebrafish and common carp……..**pp. 62 [O39]**

12.00-12.20 **Duarte D.**, Anjos C., Fatsini E., Matias D., Cabrita E.: Transcriptomic characterization of *Crassostrea angulata* cryopreserved D-larvae..**pp. 63 [O40]**

12.20-13.00 Studend Award & Closing ceremony

13.00-14.00 Lunch break

POSTER SESSIONS

SESSION 1:

BIOTECHNOLOGY AND BIOTECHNIQUES

SESSION 2:

GAMETOGENESIS

SESSION 3:

GAMETES QUALITY

SESSION 4:

FERTILIZATION AND DEVELOPMENT

SESSION 5:

GAMETE BIOLOGY

SESSION 6:

GAMETE STORAGE AND CRYORESERVATION

PLENARY LECTURE

[O1] CHROMOSOMALLY MANIPULATED AND NATURALLY OCCURRED POLYPLOID AND UNISEXUAL FISH: LESSONS FROM THE DOJO LOACH (*MISGURNUS ANGUILLICAUDATUS*)

Katsutoshi ARAI*

Professor Emeritus, Hokkaido University

*Presenting author (misgurnusclone@gmail.com)

Chromosome manipulation is a system of techniques to control numbers and combinations of conspecific and heterospecific chromosomes and it includes elevation of ploidy by inhibiting polar body release and cleavage as well as gyno- and androgenesis triggered by fertilization with genetically inactivated gametes. Chromosome manipulation itself was already investigated in the early 20th century by experimental embryologists with amphibians, but biological and aquaculture-oriented studies in fish became active in 1980's, although pioneer works existed in 1950's and 60's.

Induced triploids often showed sterility and energy reallocation from maturation occasionally resulted in growth outperformance. Triploidization was also effective to improve survival capacity in inviable hybrids. Induced gyno- and androgenesis were useful to establish unisexual population as well as to estimate sex-determination system. When gyno- and androgenetic doubled haploids were produced, clonal strains could be established by the second cycle of gyno- or androgenesis of completely homozygous gametes of doubled haploids, followed by chromosome duplication. However, production of tetraploids was very difficult.

In dojo loach, Japanese wildtype is gonochoristic diploids (2n = 50), but natural tetraploids are found in market samples (and in China). Since their induced gynogenetic progeny were viable without any chromosome duplication, they are not concluded to be evolutionary tetraploids, but genetic tetraploids with four sets of chromosomes (4n = 100). As tetraploids generate 2n gametes, cross-breeding followed by inhibition of 2nd polar body release (PBI) produced fertile hexaploid (6n = 150). Cross breeding 4n x 2n produced triploids (3n = 75) and females laid 3n eggs and 1n eggs, while males were sterile. PBI after 4n x 2n produced pentaploids (5n = 125), which females laid 2n eggs, while males had 2.3n sperm.

In certain area of Japan, clonal lineages exist and they are considered hybrid origin between genetically different ancestors. These clonal diploids produce isogenic 2n eggs which develop by gynogenesis to maintain clonal lineages, but such 2n eggs accidentally incorporate 1n sperm nucleus to develop triploids. Unreduced 2n eggs (sperm in sex-reversed clonal males) with isogenic genotypes are produced by premeiotic endomitosis to assure sister chromosome pairing. Clone-origin triploid males are sterile, whereas triploid females lay 1n eggs by meiotic hybridogenesis. Altered constitution of chromosome sets due to hybridization and polyploidization are supposed to trigger expression of atypical reproduction such as unreduced gametogenesis, spontaneous gynogenesis and meiotic hybridogenesis.

Keywords: polyploidization, gynogenesis, androgenesis, sterility

ORAL SESSIONS

SESSION 1:
BIOTECHNOLOGY AND BIOTECHNIQUES

Chairman: Martin Pšenička & Elvira Fatsini

[O2] ENHANCEMENT OF ZEBRAFISH GAMETE PRODUCTION IN A BIG BODY SIZED GIANT DANIO BY GERM CELL TRANSPLANTATION

Rigolin NAYAK[1]*, Roman FRANĚK[1], Radek ŠINDELKA[2], Martin PŠENIČKA[1]

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[2]Laboratory of Gene Expression, Institute of Biotechnology, BIOCEV, Prumyslova 595, 252 50 Vestec, Czech Republic

*Presenting author (rnayak@frov.jcu.cz)

Zebrafish *(Danio rerio*) has been predominantly used as a model species to study developmental biology, toxicological studies, and molecular genetics. However, their small size limits the quantity of gamete production, which could be challenging for the studies focusing only on the gametes, such as sperm cryopreservation and the genetic studies (not considering pooling the samples from different individuals, in case the study requires maintaining the individual genotype). Moreover, zebrafish become phenotypically male after endogenous germ cell depletion. The resurrection of cryopreserved germ stem cells through sterile zebrafish could only produce donor-derived sperm. Considering these limitations, we have come up with the idea to boost the zebrafish fecundity via giant danio surrogate (*Devario aequipinnatus),* a closely related species to zebrafish. Our research is also designed to study whether giant danios could become female after endogenous germ cell depletion. If yes, we will be able to produce donor-derived oocytes of zebrafish for the first time. Giant danio is easy to raise and breed. The adult giant danios could produce ~ 2000 eggs and ~6µl of semen (data derived from the spawning experiment) which is many times more than zebrafish. In addition to the gamete enhancement, this research will provide another possibility to preserve the germplasm of many mutant zebrafish lines, which is now only possible by sperm cryopreservation.

We have successfully standardized the method of producing sterile giant danio by blocking dead-end mRNA translation by antisense-morpholino oligonucleotide (MO) and achieved the optimum parameters for triploid production with maximum survival. The gonad of sterile recipients from the MO treated group was observed to have a testes-like structure. The qPCR results showed a higher sox9a expression similar to male controls and lowered cyp19a1a expression, which inferred that giant danios are also males without germ cells. However, the triploids are still needed to be analyzed after their maturation. The transplanted spermatogonia from vas:EGFP zebrafish successfully colonized the giant danio gonad, apparent by the observed GFP-positive cells under the fluorescence microscope. The reproductive characteristics of donor-derived gametes still need to be examined after the maturation of germline chimera.

Keywords: germ stem cell, surrogate production, spermatogonia

[O3] CRYOPRESERVATION OF RAINBOW TROUT GERMINAL STEM CELLS: APPLICATION IN AQUACULTURE TO RESTORE BOTH SPERMATOZOA AND OOCYTES FROM VALUABLE GENOTYPES

Anne-Sophie GOUPIL[1], Stéphanie KICA[1], Tina TERRENNE[2], Lionel GOARDON[2], Alexandra DEPINCE[1], Charlène LABESSE[1], Jean-Jacques LAREYRE[1], **Catherine LABBÉ[1]***

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*Presenting author (catherine.labbe@inrae.fr)

Cryobanking of fish genetic resources relies on sperm cryopreservation only, as cryopreservation of oocyte and embryos is not possible in these species with telolecithal eggs. This means that when the preserved genotype is to be restored, thanks to fertilization of plain eggs with the valuable cryopreserved spermatozoa, the offspring will bear only 50 % of the genotype of interest.

A new cellular type is able to meet this limitation: the germinal stem cells (GSCs), present in suitable quantities in immature fish gonads. It was shown previously that fish GSCs injected into recipient fries reach the gonads and are able to undergo gametogenesis to produce functional gametes. Additionally, these cells are bipotent, meaning that whatever the sex of the donor, the grafted germinal stem cells will develop in eggs if the recipient is a female, and in spermatozoa if the recipient is a male. Cryopreservation of these germinal stem cells will allow that grafted thawed GSCs ultimately produced egg, enabling fertilization with cryopreserved sperm of the same genotype.

A method for germinal stem cells cryopreservation was set up in rainbow trout, taking advantage of previous works from the fish community and considering the constraints of cryobanking this new material. We present and comment here the strategies leading to the final procedure that was adopted: the extenders are devoid of animal products, thawed cell quality was validated, and their ability to colonize recipient gonads was demonstrated. The whole procedure was tested at the INRAE PEIMA experimental farm, in order to evaluate the feasibility of this strategy in field conditions. This allowed us to determine the constraints and advantages of GSCs cryopreservation for the restoration of an entire genotype in rainbow trout.

Keywords: Oncorhynchus mykiss, grafting, vitrification, cryobanking, field application

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[O4] MOLECULAR BIOLOGY TOOLS FOR STERILE SALMON PRODUCTION

Irfan A. BHAT[1], **Milena DUBIEL[1]***, Zophonías O. JÓNSSON[1], Eduardo RODRIGUEZ[2]

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Atlantic salmon (*Salmo salar* L) is an economically important species which contributes a significant share in the marine aquaculture production of many nations. Several challenges have arisen with the expansion of the industry, attempts to reach new markets, and increase production to meet the growing demand.

Early sexual maturation is a major problem for salmon producers; this process is energetically demanding, which is reflected in adverse effects on body size, feed conversion rates, health and fillet quality. The timing of sexual maturation is a complex phenomenon involving a significant genetic component as well as environmental factors such as light abundance, temperature and food intake. Identifying genetic markers linked to delayed sexual maturation in our salmon populations will enable the implementation of selective breeding for this trait, which is not only a reliable method of delaying maturation but is also more beneficial to the welfare of the farmed stocks, as well as it decreases the risk of introgression between farmed escapees and wild stocks.

The outcome of this research will allow us to select for late-maturing or totally sterile, gonad-less salmon. A novel approach to achieve this "molecular" sterility is to produce germ cell-free salmon, which can be accomplished by knocking down or knocking out the *dead-end* (*dnd*), *vasa*, *nanos3* and *piwi* genes using antisense oligonucleotides, microRNAs and CRISPR-Cas9.

We performed a series of microinjections of antisense construct into freshly fertilized eggs and monitored the expression of the genes involved in gamete formation at various developmental stages. Our preliminary results suggest that the antisense oligos' delivery affects gene expression related to gamete formation and maturation.

Keywords: antisense oligos, CRISPR/Cas9, sterility, primordial germ cells

[O5]

TETRAPLOIDIZATION RECOVERS FERTILITY IN STERILE INTER-GROUP HYBRID MALES OF DOJO LOACH

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Hybridization often causes alterations in meiosis, which results in sterility and unreduced gametogenesis. Dojo loach, *Misgurnus anguillicaudatus*, distributed in Japan, comprises two genetically divergent populations (group A and B). In inter-group diploid hybrids in dojo loach, females were fertile and spawned haploid and diploid eggs, whereas males showed sterility with sperm dysfunction. In contrast, natural clonal dojo loach with a hybrid origin between two groups produced diploid eggs and sperm in females and sex reversal males, respectively, because tetraploid germ cells formed by genome duplication make it possible to undergo normal process of meiosis. In this study, we successfully recover fertility in inter-group hybrid males by tetraploidization.

Inter-group diploid hybrids (DiH) were produced by artificial fertilization between eggs from group B and sperm from group A. Tetraploid hybrids (TeH) were induced by heat-shock treatment in the fertilized eggs at 41.4 °C for 2 min at 22 min post fertilization. The DiH and TeH showing secondary sexual characteristics were injected with hCG for spermiation. Ploidy, motility and area of sperm head from the DiH and TeH were measured by flow cytometry, CASA and microscopic images, respectively. Fertility of sperm and viability of the resultant progeny from TeH were examined by androgenesis induced by UV irradiated generically inert eggs.

Mature TeH males produced diploid sperm, whereas semen of DiH males contained haploid, diploid and tetraploid sperm-like cells. The diploid sperm of TeH and the sperm-like cells of DiH showed significant low motility compared to haploid sperm of wild-type diploids. Average sperm head size of TeH was almost 1.5 times larger than that of wild-type diploids. Androgenetic progeny derived from diploid sperm of TeH developed into viable diploid larvae, and some of them grew normally as in triploid progeny in control crosses. In conclusion, inter-group hybrids recovered their fertility by tetraploidization, which makes it possible to undergo meiosis normally with pairing between duplicated homologous chromosomes.

Keywords: allotetraploid, synapsis, unreduced gamete, amphidiploid

Acknowledgements: Supported by Grants-in-Aid from JSPS (Japan Society for the Promotion of Science) KAKENHI Grant Numbers JP21H02278.

[O6]

QUALITY OF RAINBOW TROUT EGGS AND EFFICIENCY OF ANDROGENESIS AND GYNOGENESIS

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Androgensis and mitotic gynogenesis result in generation of fully homozygous Doubled Haploid individuals in a single generation. DHs have been used in the selective breeding programs, in studies concerning phenotypic consequences of the recessive alleles and to evaluate impact of sex chromosomes on the early ontogeny. Moreover, use of DHs for NGS approach radically improves *de novo* assembly of the genomes. Even though, reduced survival of the doubled haploids limits application of androgenotes and gynogenotes in aquaculture. High mortality of DHs may be only partly explained by the expression of recessive traits. Observed inter-clutch variation in survival of DH rainbow trout embryos developing in eggs originating from different females made to take a closer look on the quality of eggs used during induced andro- and gynogenesis. Here, recently provided results concerning morphological, biochemical, genomic and transcriptomic characteristics of rainbow trout eggs showing high and low competence for androgenesis and gynogenesis are reviewed. Eggs with the highest developmental competence for androgenesis are morphologically (size of eggs and distribution of the lipid droplets) similar to those that have not got such potential but they exhibit increased activity of the antioxidant enzymes such as SOD, CAT and GPx [1]. Eggs with increased survival of gynogenotes also showed increased expression of genes that are associated with early embryogenic development, cell survival, migration and differentiation, triglyceride metabolism and biosynthesis of polyunsaturated fat, and senescence and aging, among others [2]. Moreover, analysis of the egg transcriptome confirmed that bigger differences in the maternal gene expression are between eggs from the different clutches than between eggs from the same clutch but treated and non-treated with the pressure shock utilized for diploidization of the andro- and gynogenetic zygotes [3]. Taking into account, mentioned above information, a strong maternal effect on the chromosome set manipulations can be assumed.

Keywords: transcriptome, antioxidant enzymes, egg developmental competence

Acknowledgements: Supported by the National Science Centre (NCN) in Poland (grant number 2020/39/B/NZ9/00865).

References:

[1] Polonis M, et al. (2021). Inter-clutch egg differences and androgenesis in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). *Oceanol Hydrobiol Stud.*

[2] Ocalewicz K, et al. (2020). Preliminary identification of candidate genes related to survival of gynogenetic rainbow trout (*Oncorhynchus mykiss*) based on comparative transcriptome analysis. *Animals*.

[3] Gurgul A, et al. (2018). Transcriptome analysis of rainbow trout (*Oncorhynchus mykiss*) eggs subjected to the high hydrostatic pressure treatment. *Int J Genomics*.

[O7]

OPTIMIZATION OF CULTURE MEDIA FOR *IN VITRO* MATURATION OF COMMON CARP OVARIAN FOLLICLES

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Successful stimulation of *in vitro* oocyte maturation (IVM) highly depends on proper hormonal stimulation and optimal culture conditions. For many fish species, the most potent IVM inducer is 17α,20β-dihydroxy-4-pregnen-3-one (DHP). However, the composition of culture media used in these protocols vary between simple salt solutions, such as Hank's Balanced Salt Solution (HBSS) and Cortland's medium, to pre-defined complete media with additives, namely Leibovitz (L-15) medium. Here we report the optimization of an *in vitro* system to induce and support maturation of ovarian follicles isolated from common carp (*Cyprinus carpio* L.). After isolation, fully grown, postvitellogenic carp ovarian follicles (opaque, 1.19±0.1 mm) were placed into appropriate culture media –HBSS, Cortland's or 90% L-15– supplemented with antibiotics (pH 7.6, 290±5 mOsm/kg). Maturation was induced with DHP, and its progress evaluated by scoring the percentage of follicles that underwent germinal vesicle breakdown (GVBD) and ooplasm clearing. Compared to HBSS, both Cortland's and 90% L-15 medium supported significantly higher maturation rates (> 80% GVBD) after 10 hours of incubation. Further optimization of 90% L-15 medium by adjusting the pH to slightly alkaline values of 8.5 markedly enhanced the rate of DHP-induced maturation. This outcome was improved with incorporation of exogenous protein sources, namely 0.1% Bovine Serum Albumin (BSA) or 10% Fetal Bovine Serum (FBS). The highest rate of GVBD (93%) was present in the 90% L-15 medium with 0.1% BSA, at pH 8.5. There was no spontaneous GVBD in any of the control groups without DHP. The responsiveness of ovarian follicles was in a clear in a dose-dependent manner, whereby DHP concentration of 1 μg/ml elicited the highest maturation rate. However, even prolonged incubation in any of the treatment groups did not lead to ovulation of the matured oocytes, which implies that DHP alone is not sufficient to induce this process under present conditions. Therefore, effects of other potential maturation- and ovulation- inducing substances should be assessed to further confirm the developmental capacity of *in vitro* matured carp oocytes.

Keywords: oocytes, GVBD, Cyprinus carpio, Leibovitz L-15, DHP, IVM

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SESSION 2:
GAMETOGENESIS

Chairman: Juan Asturiano & Joanna Nynca

[O8]

KISSPEPTIN TREATMENT INDUCED REPRODUCTIVE TRAITS DURING PUBERTY AND ALTERED CHEMICAL COMMUNICATION IN SENEGALESE SOLE

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Senegalese sole (*Solea senegalensis*) is a highly valuable species for European aquaculture, still facing reproductive constrains in F1 breeders (born and reared in captivity). Kisspeptin, a crucial regulator of puberty and reproduction onset, showed a high potential to be used as a hormonal treatment in this species, as a single injection activated the BPG axis of F1 breeders at different levels [1]. In the present work, it was pretended to test this same effect in juveniles as an accelerator of puberty, and to study a possible impact at the level of chemical communication in adult breeders. In a first trial, prepubertal Senegalese sole (approximately 1.5 year old), were treated with either a single injection of KISS2 decapeptide at a dose of 250 µg/kg body weight, or PBS as a placebo control. Blood plasma was collected at different moments (before, 4 hours, 2 and 4 days after treatment) for sex steroids determination (Testosterone, T and Estradiol, E2 in females; T and 11-Ketotestosterone, 11-KT in males) by ELISA. Gonads were excised at 4 days post treatment, to determine gonadal maturation stage by histology. Results revealed higher T levels in both sexes after kisspeptin treatment, with treated females also having an E2 increase trend, however non-significant. Histology analyses in male testis revealed increased spermatids and reduced spermatocyte percentage in the cortical region of treated fish, indicating a more advanced stage of the gonad. These results evidenced a positive effect of kisspeptin treatment on sole puberty, reinforced by studies on other species. In a second experiment, adult sole breeders were treated in similar way, to test kisspeptin effect on their chemical communication. Urine samples from both groups were run to a LC-MS analysis to identify relevant compounds, and an EOG analysis, comparing their effect on sole olfactory epithelium. Two compounds were found in higher concentration in the treated sole's urine, both theorized to be pheromones, given that treated urine had increased potency on olfactory responses for both sexes. The results of both trails reinforce the previously described potential effect of this hormone, to be used as a hormonal treatment in sole.

Keywords: KISS2 decapeptide, Solea senegalensis, gonadal maturation, EOG analysis

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References:

[1] Oliveira CCV, et al. (2020). Kisspeptin Influences the reproductive axis and circulating levels of microRNAs in Senegalese sole. *Int J Mol Sci.*

[O9]

INSIGHTS ON REPRODUCTIVE GENDER SPECIFIC TOXICITY INDUCED BY GLYPHOSATE CHRONIC EXPOSURE IN *DANIO RERIO* ADULTS

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Glyphosate is the active compound of the herbicide formulation Roundup® and is commonly used for weed control in crops, gardens and municipal parks. Despite no action against non-target organism was supposed, its wide application increased concern on both wildlife and humans toxicity. An exposure to 700 µg/L concentration of Glyphosate, the Maximum Concentration Level (MCL) assessed by EPA in drinking water, was carried out on male and female adult zebrafish, while a second group was exposed to a volume of Roundup® containing glyphosate at 700 µg/L and the results were compared to those of a control group to assess reproductive toxicity of this xenobiotic. Shortly after the addition of the herbicide, fish exposed to Roundup® died while those exposed to glyphosate were sacrificed after a 28 day and tissue were sampled. Metabolomic analysis evidenced a disruption of hepatic metabolism, an increased stress and inflammatory response in female and the disruption of oxidative stress response in male, suggesting a sex-specific toxicity. Considering the hepatic alterations, the aim of this study was to investigate the effects at gonadal levels hypothesizing a possible impairment at the reproductive level. In particular, in male, histological results clearly showed the alteration of gametogenesis, with testis of fish exposed to glyphosate presenting an increase count of Spermatogonia A, B and spermatids and a decrease in spermatozoa. On the other hand, previtellogenic-, vitellogenic- and mature follicles number showed no differences between control and treated ovaries. A deeper investigation at transcriptional level was made to gain evidence regarding glyphosate hormonal behavior. Thus, the expression of genes involved in gonadal steroidogenesis, differentiation and apoptosis was analyzed, suggesting an estrogenic and antiandrogenic effect, as well as a sex-specific effect of this widely spread pollutant.

Keywords: herbicide, oogenesis, spermatogenesis, zebrafish, vitellogenins

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[O10]

HIPPO PATHWAY-MEDIATED REGULATION OF MICROPYLE FORMATION BY MICRO RNA 202 (MIR-202) IN THE FISH OOCYTE

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Micro RNA 202 (miR-202) is predominantly expressed in gonads in fish and vertebrates. In medaka (*Oryzias latipes*) miR-202 is plays a key role in female reproductive success [1]. Eggs originating from *miR202* -/- knock out females mated with mutant males exhibit a dramatically reduced early embryonic developmental rate [1].In this context, the aim of our study was to decipher underlying mechanisms to understand how miR-202 regulates fertility. We show that sperm does not enter the egg originating from -/- females despite the presence, in most cases, of a micropyle (i.e., the channel through which the spermatozoa enters the egg at fertilization) on the chorion. We investigated micropyle presence and functionality using direct observations and scanning electron microscopy. We provide evidence indicating that the micropylar precursor cell (MPC) undergoes abnormal differentiation during follicular growth, ultimately leading to non-functional micropyle and impaired fertilization. Taz, the main effector of Hippo pathway signaling, is required for MPC formation in zebrafish [2,3]. Here we show that several genes of the Hippo pathway are dysregulated in the ovary of mutant miR-202 -/- fish including genes that are predicted targets of miR-202. Using RNA-seq analysis of early-vitellogenic and late-vitellogenic ovarian follicles sampled from wild-type and mutant females, we were able to identify dysregulated genes that further support the hypothesis of abnormal MPC differentiation. When comparing differentially expressed genes at EV stage with *in silico* predicted targets of miR-202-5p we identified strong candidates for being the main target(s) of miR-202 in the MPC, including members of the Hippo pathway. Genome editing-based investigations to demonstrate the biological relevance of such targets through removing the miR-202-5p target site in the 3'UTR region are currently in progress. Together, our data suggest that miR-202 regulates micropyle formation through the modulation of the Hippo pathway in the ovary.

Keywords: medaka, micropylar precursor cell, scanning electron microscopy, RNA-seq analysis, ovary

Acknowledgements: Supported by Agence Nationale de la Recherche (MicroHippo - ANR-CE20-0023).

References:

[1] Gay S, et al. (2018). MiR-202 controls female fecundity by regulating medaka oogenesis. *PLoS Genet.*
[2] Dingare C, et al. (2018). The Hippo pathway effector Taz is required for cell morphogenesis and fertilization in zebrafish. *Development.*
[3] Yi X, et al. (2019). The effector of Hippo signaling, Taz, is required for formation of the micropyle and fertilization in zebrafish. *PLoS Genet.*

[O11]

FSH REGULATES THE PROLIFERATION OF EMBRYONIC-LIKE GERM STEM CELLS IN ADULT ZEBRAFISH TESTES

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A novel subpopulation of pluripotent stem cells, named embryonic-like stem cells (ELs), has been recently reported among spermatogonial stem cells in humans and mice. Furthermore, it has been shown that ELs in testes and ovaries express FSHR, and FSH has a direct effect on these cells. In this study, we sought to investigate whether ELs were present in zebrafish testes. To address our aims, expression analyses (RT-qPCR) of genes involved in pluripotency were carried out during zebrafish embryonic stages and the culture of testicular explants incubated or not with recombinant zebrafish Fsh (rzfFsh). To further identify the pluripotent markers and Fshr, immunofluorescence, western blot or flow cytometry were employed on wild-type or Fshr:eGFP zebrafish reporter lines. Finally, RNAseq libraries were produced from total RNA extracted from zebrafish testicular explants cultivated with trilostane (an inhibitor of sexual steroid production) in presence or absence of rzfFsh (100 ng/mL). We first demonstrated that the selected pluripotent genes, *pou5f3*, *nanog* and *nanos3*, showed higher expression levels at the blastula stage, and later, mRNA levels were significantly down-regulated over the gastrulation. Furthermore, we showed that Pou5f3, Nanog and Nanos3 were found among the different generations of spermatogonia although their staining pattern varied depending on the spermatogonial development in adult testes. The pluripotent markers were expressed at higher levels in early spermatogonia (type A undifferentiated (A_{und}) and differentiated (A_{diff}) spermatogonia) compared to type B spermatogonia, and no longer detected in meiotic and post-meiotic germ cells. Using a specific antibody, we observed that Fshr was expressed in somatic cells and in A_{und} and A_{diff}. We further evaluated whether the selected pluripotent genes were regulated by Fsh. Similar to mammals, we found that Fsh increased *pou5f3* mRNA levels, while *nanog* and *nanos3* were down-regulated after 7 days of Fsh exposure. RNAseq libraries also showed a deregulation for many mediators of the stem cell signaling pathway in the testes cultivated with Fsh. Finally, we observed that a transgenic zebrafish line carrying the GFP reporter gene under the control of a proximal promoter fragment of the *fshr* gene showed high GFP expression levels not only in somatic cells, but also in A_{und} and A_{diff}. Altogether, our data indicate the existence of Fsh-dependent proliferating ELs in adult zebrafish testes.

Keywords: stem cell, pluripotency, germinal niche, endocrine regulation, fsh

Acknowledgements: Supported by FAPESP (20/03569-8).

SESSION 3:

GAMETES QUALITY

Chairman: Julien Bobe & Taina Rocha De Almeida

[O12]

EFFECT OF *MORINGA OLEIFERA* SUPPLEMENTED DIET ON THE SPERM QUALITY OF MALE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Moringa oleifera leaves (MOL) were added to the broodstock trout feed as a supplement to examine the influence on the reproductive performance of male rainbow trout (*Oncorhynchus mykiss*) broodstock. Moringa is a superfood rich in protein and vitamins native to Bangladesh and the Indian subcontinent. Moringa leaves were collected from Narsingdi, Bangladesh and air-dried to use in the diet. Thirty-six individuals of 3+ year male rainbow trout (1786.36±212.14 gr) under four groups (MOL-1/control, MOL-2, MOL-3, and MOL-4) with three parallels each were used in 12 circular fiberglass tanks. Moringa leaves were included in the diets at different levels: 0% (MOL-1), 4% (MOL-2), 8% (MOL-3), and 16 % (MOL-4), and fed twice a day for 18 weeks. Sperm and eggs were collected by abdominal message using the dry stripping method during the spawning season. A computer-aided sperm analysis (CASA) system (CEROS II: Hamilton-Thorne, Beverly, MA, USA) connected to a CX41 microscope (Olympus, Japan) was used to analyze the sperm motility parameters at room temperature. Leja 4 chamber slide, 20 µm deep (Leja Products, Netherlands), was used in the CASA system. Sperm Volume (ml), Sperm pH, Seminal Plasma Osmolality (mOsm/kg), Spermatozoa Density (10^9/ml), Motility Duration (sec), Total motility, and Sperm motility parameters [Average Path Velocity (VAP) (µm/s), Linear Velocity (VSL) (µm/s), Curvilinear Velocity (VCL) (µm/s)] were investigated. Regarding gamete quality between groups, fertilization success, eyeing, and hatching rates were calculated as percentages. Fertilization rates were highest (87.63±8.57%) in the MOL-3 group and lowest (82.37±7.01%) in the MOL-2 group compared to other groups. The lowest eyeing and hatching rates were found in the MOL-1 group at 76.72±7.15% and 69.34±8.39, respectively; the highest eyeing and hatching rates were found in the MOL-3 group at 83.89±8.36% and 77.42±7.53%, respectively. Therefore, moringa can be recommended as a supplement diet for rainbow trout broodstock.

Key words: trout feeds, fertilization success, CASA, sperm motility, hatching rate

Acknowledgment: Supported by Scientific Research Projects Coordination Unit of Istanbul University. Project numbers: FDK-2021-38147 and FBG-2018-31504.

[O13]

PREDICTING THE EGG QUALITY BASED ON THE OOCYTE DIAMETER AND *IN VITRO* MATURATION IN DOMESTICATED PIKEPERCH *SANDER LUCIOPERCA*

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Unlike in wild fish, final oocyte maturation (FOM) indicators were not suitable for captive pikeperch grown on artificial diets. Therefore, our work aimed at monitoring the oocyte diameter over the last several months of the pikeperch reproductive cycle and using the techniques of *in vitro* biological tests of FOM (IVM). In the first year, we evaluated the minimal oocyte diameter of responsiveness to hormonal stimulation in indoor-reared females. Further on, we evaluated the oocyte diameter in preseason reproduction at the early stage of oocyte maturation competence (OMC). The third trial was devoted to monitor the oocyte diameter from the stage of late vitellogenesis until the start of the natural reproductive season. Finally, IVM techniques were used to evaluate its relationship with *in vivo* FOM dynamics. The first trial led to the conclusion that overgrowing 900 µm oocyte reaches the initial OMC state when fish become responsive to salmon gonadotropin releasing hormone analog stimulation. In the second trial, this minimal oocyte diameter border was confirmed and it was also shown that in preseason oocyte size directly correlates with egg quality. The outcome of the third trial showed inter-individual maturational differences in fish among common broodstock. Variability in dynamics of oocyte maturation was visible already in autumn. Likewise, this trial showed that the seasonal state of higher OMC in faster-growing oocytes leads to improper FOM visible in oil globule fragmentation and malformation of the newly hatched larvae. Finally, the potential of IVM as the evaluator of the OMC was shown in the preseason as correlation was found with the oocyte diameter and *in vivo* latency time (period from hormonal stimulation to ovulation). Overall our research showed individuality in domesticated broodstock visible already in vitellogenesis leading to earlier OMC compared to wild fish. Likewise, faster maturing fish appear to be sensitive to external hormones in case of more advanced OMC state. Therefore, our future research will aim at developing IVM protocols for evaluation of the optimal OMC state for artificial reproduction.

Keywords: artificial reproduction, vitellogenesis, final oocyte maturation, oocyte maturation competence

Acknowledgements: Supported by the National Research, Development and Innovation Fund of Hungary (grants PD-139053 and K138425).

[O14]

UNPREDICTABLE CHRONIC STRESS ALTERS BEHAVIOUR, SPERM QUALITY AND NMD PATHWAY IN ZEBRAFISH

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Stress potentially affects several biological functions including reproduction. Good practice for fish care and housing includes optimal feeding regimes, adequate water quality and appropriate animal densities. However, there are many other practices that can act as potent stressors and can modify animal behavior and eventually reproductive performance. Novel tank test (NTT) is a well-established method to study zebrafish anxiety levels [1]. In this study we analyzed the effects of exposing zebrafish to an unpredictable chronic stress during one spermatogenic cycle evaluating fish behavior, gene expression, testicle histology and gamete quality.

All animals were standard manipulated according to the Guidelines of the European Union Council (2010/63/EU), following Spanish regulations (RD/2013) for the use of laboratory animals Animal. Experiments were approved by University of León Ethical Committee (OEBA-ULE-009-2020). Fish were labelled with visible implant elastomers (VIE tagging) for individual tracking. The experimental animals (S+) were kept in 3 tanks (10 fish per tank). Fish included in the S+ group were exposed to different stressors twice a day in order to guarantee an unpredictable exposure. Control fish (CTRL) (3 tanks, 10 fish per tank) were kept under normal housing conditions. After the stress exposure (t=21d), behavior analysis was performed by NTT using EthoVision XT (version 16, Noldus) Software, total RNA was extracted from testes for gene expression studies, testicular samples were extracted and fixed for histology evaluation and 3 males per tank were squeezed for sperm extraction and evaluation using CASA system.

Swimming time in the upper tank zone (correlated to a lower-anxiety behavior) was significantly reduced ($p=0.0315$) in those fish exposed to stressors (% mean values: 18.79 ± 3.631 in CTRL vs 8.601 ± 2.395 in S+) demonstrating that the exposure of fish to an unpredictable chronic stress for one cycle of spermatogenesis significantly increased their anxiety levels. qPCR analysis demonstrated that several genes related to the Nonsense-Mediated-Decay Pathway were significantly downregulated in the testicle. Interestingly, statistically significant decreases in total motile cells ($p=0.0122$), progressive cells ($p=0.0003$) and fast cells ($p=0.0211$) were also observed in the experimental group (S+) after stress exposure. This study demonstrated that stress does not only affect fish behavior increasing anxiety levels, but also has an important impact at molecular level and affects sperm quality being an important factor disrupting fish reproduction in fish species.

Keywords: zebrafish, stress, gene expression, behavior, sperm quality

Acknowledgments: PID2019-108509RB-I00 project (Ministerio de Ciencia e Innovación), FCJ2018-037566-I grant.

References:

[1] Levin ED & Cerutti DT. Behavioral Neuroscience of Zebrafish. Methods of Behavior Analysis in Neuroscience (CRC Press/Taylor & Francis, 2009).

[O15]

SPECIES-SPECIFIC MELATONIN PATTERNS IN FISH SEMINAL PLASMA

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Melatonin is a clock-hormone mainly produced by the pineal organ, but its peripherical targets and physiological roles in the overall organism are still under study [1]. In mammals, melatonin was found to be present in seminal plasma and to have a positive correlation with gamete quality and antioxidant activity [2]. As the best of our knowledge, there is no information regarding melatonin in fish seminal plasma. Our study aimed to identify the possible presence of melatonin in seminal plasma from three different teleost species. European Seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) captive-reared broodstocks, and both F1 and wild Senegalese sole (*Solea senegalensis*) breeders were sampled during the reproductive season. Both blood and seminal plasma were collected at mid-day (ML) and mid-night (MD), and melatonin concentration was determined by radioimmunoassay (IBL RIA kit, Germany). In all species analyzed, blood plasma melatonin peaked at MD, as expected, but regarding seminal plasma melatonin, the obtained results were different. Seminal plasma melatonin was not found in European seabass; in gilthead seabream it was found only at MD, and in Senegalese sole F1 and wild males it displayed different patterns. The higher values were obtained in gilthead seabream MD samples, with an average of 808 pg/mL of melatonin in blood plasma and 21 pg/mL in seminal plasma. In conclusion, it was proven the existence of species-specific melatonin patterns in fish seminal plasma, and its variability between day and night. Further investigation is needed to understand the physiological role of seminal plasma melatonin.

Keywords: radioimmunoassay, seminal plasma, teleost species, captive-reared, wilds

Acknowledgements: Supported by the Portuguese Foundation for Science and Technology, through a PhD (SFRH/BD/148280/2019 to F.F.), contract DL 57/2016/CP1361/CT0007 to C.C.V.O., and the projects ReproF1 (Programa Operacional Mar2020, MAR-16-02-01-FMP-0059) and SpermAntiOX (UIDB/04326/2020 and PTDC/CVT-CVT/4109/2020).

References:

[1] Reiter RJ, et al. (2016). Melatonin as an antioxidant: under promises but over delivers. *J Pineal Res.*
[2] Casao A, et al. (2010). Melatonin prevents capacitation and apoptotic-like changes of ram spermatozoa and increases fertility rate. *J Pineal Res.*

PLENARY KEYNOTE

[O16]

IT TAKES TWO TO TANGO AND IF YOU ARE A FISH OOCYTE YOU WILL ALSO NEED ENOUGH RIBOSOMES

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One of the energetically most demanding processes in a cell is protein synthesis and fish oocytes know about this. A good oocyte needs to accumulate enough tRNAs and be ready for fast production of ribosomes so early embryo protein production can be initiated in case of fertilization. While the transcription of the precursor of 5.8S, 18S and 28S rRNAs is regulated by RNA polymerase I (Pol-I), 5S rRNA and tRNAs are transcribed by Pol-III. Pol-III in turn, is regulated by activating transcription factors (Gtf3a, b and c) and the inhibitor Maf1. We have demonstrated in more than 10 teleost fish species that 5S rRNA and tRNAs are so highly expressed in oocytes that they serve as markers of oocyte presence in gonads. This includes intersex testes in fish exposed to xenoestrogens as demonstrated in mullets *Chelon labrosus* from polluted estuaries in the Southern Bay of Biscay. They are the main transcripts in previtellogenic oocytes while 5.8S, 18S and 28S rRNA begin to accumulate in oocytes during secondary growth. Therefore, the dynamics of Pol-III and Pol-I transcripts can be used to quantitatively identify the ovarian developmental stage in teleosts. A simple electrophoretic analysis of gonadal total RNA and calculation of 5S rRNA/18S rRNA and tRNA/5.8S rRNA indices in Bioanalyzer electropherograms suffices for such staging.

How is Pol-III regulated in fish ovaries? We have found that *gtf3a* gene is duplicated in teleost genomes and *gtf3ab* has been subfunctionalised showing ovarian exclusive expression, especially during previtellogenesis. Gtf3b instead is a multipeptidic transcription factor and teleosts show duplications of all their coding genes (*brf1a* & *b*, *brf2a* & *b* and *bp1a* & *b*). Additionally, a duplication in the inhibitor *maf1* is present in zebrafish but not in other teleost genomes. Orthologs for all such genes have been sequenced in *C. labrosus*, our pollution sentinel species, but only one *maf1* gene. PCR analyses have revealed transcription of these genes in all mullet and zebrafish tissues, including gonads. qPCR analyses will allow to decipher whether any of the identified paralogs displays higher ovarian than testicular transcript levels, compatible with the transcription pattern of 5S rRNA, tRNAs and *gtf3ab*. We hypothesize that good quality fish oocytes accumulate molecules that will allow rapid assembly of functional ribosomes and rapid protein synthesis so early embryo development can be sustained after fertilization. Therefore, molecules participating in protein synthesis can constitute molecular tools to identify good quality oocytes in fish. Without doubt these markers are useful in the identification of intersex fish consequence of exposures to xenoestrogens downstream wastewater treatment plants. Ribosomes are needed for the tango!

Keywords: oocytes, 5S rRNA, tRNA, RNA polymerase III, Chelon labrosus, intersex

Acknowledgements: Supported by Basque Government (IT1302-19), Spanish MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

SESSION 4:

FERTILIZATION AND DEVELOPMENT

Chairman: Oliana Carnevali & Marta Lombo

[O17]

THE GROWTH PERFORMANCE OF POND REARED LARVAE PROPAGATED USING CRYOPRESERVED SPERM IN COMMON CARP (*CYPRINUS CARPIO*)

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In the past 45 years, the cryopreservation of common carp sperm was intensively studied. Numerous methods were presented which achieved high efficiency in laboratory conditions and hatchery as well. However, now data is available (according to our knowledge) regarding to extensive larvae rearing at pond culture following hatchery propagation using large-scale cryopreserved sperm. The aim of our study was to test our formerly developed large-scale sperm freezing method (103 pcs of 5 mL straw, dilution ratio 1:9, extender: 250 mM glucose, 20 mM NaCl, 25 mM KCl, 1 mM $Na_2HPO_4 \times 12H_2O$, 1 mM $MgCl_2 \times 6H_2O$, 1 mM $CaCl_2 \times 2H_2O$, 20 mM Tris and 0.5% BSA, pH: 8.0±0.02) during propagation (25 mL fresh and 250 mL cryopreserved sperm per 1 kg of eggs) at hatchery conditions. Furthermore, hatched larvae were reared at traditional earthen ponds separately in two phases (pre-nursing: 3 weeks and grow-out: 16 weeks). Hatching and larvae malformation rate was recorded at the moment of hatching. Growth rate parameters (total length, average body weight) of larvae were monitored in 2 weeks interval (8 times), where survival rate was calculated at the end of the pre-nursing as well as the grow-out phase. A significantly higher hatching rate was measured by fresh (87%) in comparison with the frozen group (42%) where no significant difference was detected by the larvae malformation rate (fresh: 45%, cryopreserved: 65%). During the larvae rearing, no significant effect of freezing was observed on the growth rate. A significantly higher survival rate was found in the fresh (72%) compared to the cryopreserved group (43%) at the end of pre-nursing. In contrast, a reverse result was recorded at the end of the grow-out where a significantly higher survival rate was calculated by the cryopreserved (96%) in comparison with the fresh group (95%). In conclusion, large-scale cryopreserved sperm has the potential in the hatchery application following crucial optimization of the handling large amount of straws (103 pcs). According to the observation of the hatchery leader at the fish farm, the pond selection had a main effect on the survival rate at the end of the pre-nursing phase (more predator organisms, negative effect of pond macrovegetation etc.) by the cryopreserved group. In general, the application of frozen sperm had no negative effect on the growth rate and viability of larvae at the end of the grow-out phase.

Keywords: frozen sperm, propagation, hatching rate, pre-nursing, grow-out, survival rate

Acknowledgements: Supported by the MGEF/20-3/2021, GINOP-2.3.2-15-2016-00004, ÚNKP-21-2-1-MATE/16. Our study was co-financed by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, National Challenges Subprogramme (TKP2020-NKA-16).

[O18]

DNA DAMAGE RESPONSE IN STURGEON (*ACIPENSER RUTHENUS*) EMBRYO: LINK BETWEEN PHENOTYPE AND TRANSCRIPTOME

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The early stages of embryo development are characterized by rapid cell proliferation, growth and DNA replication. In externally fertilizing organisms the developing embryo is exposed to a variety of genotoxic stressors throughout development, which include environmental pollution and radiation. Embryos are able to minimize adverse effects of external factors and/or overcome the consequences by using stress response pathways including DNA damage repair. In the current study, the focus was made on the embryonic response to DNA damage in sterlet (*Acipenser ruthenus*). Sturgeons (*Acipenseridae*) are important in aquaculture, mainly due to production of black caviar and boneless meat. Most of sturgeon species have been classified as endangered. However, very little is known about pathways of DNA damage repair in sturgeon embryos.

In the current study we have analyzed DNA damage response in embryos of *A. ruthenus* exposed to camptothecin (CPT) and olaparib. We have evaluated the level of induced DNA damage and performed RNA sequencing and proteomics. The results of this study indicate that DDR in sturgeon embryos is stage-dependent. Further, we observed a correlation between phenotype formation and changes in transcriptomic and proteomic profiles. CPT and olaparib downregulated oxidative phosphorylation and metabolic pathways, and upregulated pathways involved in nucleotide excision repair, base excision repair, and homologous recombination. The analysis of gene expression revealed several markers of DDR and adaptive stress-response, which could be applied in toxicological studies on fish embryo. This study revealed the complexity of DNA damage response and indicate diverse roles of DNA repair genes and proteins in fish embryo development.

Keywords: fish, omics, toxicology, stress response

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[O19]

STURGEON (*ACIPENSER*) REPRESENTS THE EVOLUTIONARY TRANSITION FROM THE HOLOBLASTIC TO MEROBLASTIC CLEAVAGE PATTERN AND UNIQUE GUT DEVELOPMENT

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A vertebrate embryo's cleavage pattern is either holoblastic (complete) or meroblastic (partial). Holoblastic cleavage is thought to be ancestral to vertebrates and is most likely to occur in amphibians, mammals, and chondrosteans. Meroblastic cleavage has evolved five times in vertebrate lineages, including hagfish, elasmobranchs, coelacanths, teleosts, and amniotes. This transition is usually occurred by an increase in egg size in comparison to the lineage's ancestral state. Sturgeons' eggs are significantly larger than that of *Xenopus laevis*. Despite the variation in sizes, their embryos retain nearly characteristics the same as that of *X. laevis*. Thus, it was speculated that vegetal blastomeres of sturgeon are extraembryonic as in yolk of teleost (zebrafish) and Yolk cells of (YCs) of bichir—earliest diverged living group of actinopterygian fishes, agnathan lampreys (Petromyzontidae)—an extant lineage of jawless fishes and an *Eleutherodactylus coqui* (direct developing frog). Furthermore, the gut development pattern of sturgeon (*Acipenser*) and its evolutionary conservation was poorly understood so far.

First, we developed the robust technique for specific blastomeres inhibition of sturgeon embryos using diatoms-derived polyunsaturated aldehydes, 2, 4–Decadienal (DD; a model aldehyde for experimental studies). The sturgeon's embryos were injected with optimal DD percentage (0.01 v/v) and subsequently irradiating them by visible light (91.15 – 44.86 W m^2). Furthermore, qPCR-tomography revealed that localized pattern of maternal mRNA remained constant through animal–vegetal axis in partially cleaved embryos when compared to normal.

Second, fate-mapping of sturgeon vegetal blastomeres revealed that these blastomeres gave rise to primordial germ cells, and the rest of the descendants were vegetal YCs. Plastic section histology showed that the nuclei of YCs sharply declined as embryos developed. In addition, inhibition of vegetal blastomeres, RT-qPCR and BrdU pulse revealed that YCs become transcriptionally inactive after mid-blastula transition. Here, our results suggested that the meroblastic cleavage in actinopterygian lineage had evolved by the fusion of vegetal blastomeres, which is parallel to the closely related group, e.g., gar (Lepisosteidae), that evolved at approximately 57 million years ago.

Lastly, using histology, in-situ hybridization (HCR) and Immunohistochemistry observation, we studied the sturgeon gut development and its comparison with other taxa including holoblastic (*X. laevis*, bichir, and mice) and meroblastic (chicks, gars, and zebrafish) representatives. We found that sturgeon's endodermal cells formed the Archenteron (primitive gut) as Xenopus and bichir. However, these cells continued to proliferate lateroventrally to encompass a massive amount of yolk mass to give rise "yolk inside the gut."

In conclusion, our findings suggest that sturgeon embryo development represents a distinct transition from holoblastic to meroblastic cleavage, as well as a distinct archaic mode of gut-endoderm development.

Keywords: holoblastic cleavage, meroblastic cleavage, cleavage pattern inhibition, RNA localization, fate-mapping, extra-embryonic nutrition, gut-endoderm development

[O20]

THE INVESTIGATION OF THE DIFFERENCES IN ARTIFICIAL PROPAGATION AND LARVAE DEVELOPMENT BY FOUR DIFFERENT GOLDFISH TYPES (*CARASSIUS AURATUS*)

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Due to the origin and diversity of goldfish, it is a suitable model organism to investigate the effect of artificial selection on reproduction and larvae development. In our study, we compared the differences (the effects of artificial selection) in four phenotypically notably different varieties of goldfish (Common goldfish, Shubunkin, Black Moor, Oranda) within the framework of propagation, larval rearing and morphology studies. In our propagation test, the progressive motility of the sperm was measured and the number of eggs in 1 g was calculated. The fertilization was determined 24 hours, whereas the hatching rate was 48 hours post-fertilization. During the larvae rearing, the average standard body length, and the average body weight of the individuals were recorded at three developmental stages, after hatching, at the absorption of the yolk sac and at the one-week feeding larval stage. Furthermore, the larval malformation rate was determined in these stages. At the end of the larvae rearing, the survival rate was also calculated. Significantly lower progressive motility was measured in Black Moor (79 ± 13%) than in the other varieties. A significant difference was observed in the number of eggs in 1 g between the Common goldfish (1733 ± 606 pcs) and the Black Moor (1091 ± 69 pcs). A significantly higher fertilization rate was measured in Oranda (93%) compared to the other three types. The hatching rate was significantly lower (44%) in the case of Black Moor than in the more varieties**.** During larvae rearing, we found a significant difference between the body length of Common goldfish and Shubunkin at the stage of hatched larvae. After absorption of the yolk sac, a significant difference was recorded between Shubunkin and Oranda. In the one-week feeding larval stage, the Common goldfish and Shubunkin had significantly lower average body weight, than the Black Moor and the Oranda. At the stage of hatched larvae, a significantly higher malformation rate was found in Shubunkin (70%) and Black Moor (75%) compared to the others. At the one-week feeding larval stage, the malformation rate was significantly lower in the case of Common goldfish (5%) than in the Black Moor (60%). A significantly higher survival rate was recorded for the Common goldfish (95%) than for Shubunkin (91%) and Oranda (92%). Based on our results, clear differences were observed in the reproduction and larval development between the goldfish varieties which originated from the same population (individuals of the same age and the same environment). Furthermore, the effect of artificial selection could be recognized in both the reproductive capacity and the larvae development of the investigated goldfish types.

Keywords: goldfish, artificial selection, propagation, larvae rearing, larvae morphology

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[O21]

REPRODUCTIVE BIOLOGY OF THE EUROPEAN SARDINE (*SARDINA PILCHARDUS*) IN THE ADRIATIC SEA, AN INTEGRATED STUDY EVALUATING OVARY STRUCTURAL AND MORPHOLOGICAL INTEGRITY WITHIN AN ENTIRE REPRODUCTIVE CYCLE

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The European sardine (*Sardina pilchardus,* Walbaum, 1792) represents one of the most important small pelagic fish resources in the Mediterranean Sea and in particular in the Adriatic area where it represents the majority of landings from both large-scale and small-scale fisheries. During the last years, the fluctuation registered in the total catches of sardines in the Adriatic Sea raises the question of the population's future stability. One of the most crucial aspects of fish life cycle is the reproduction and its success on which the population growth depends and therefore its survival also as a marine resource.

This study was performed to deepen the knowledge of sardines' reproduction cycle with a particular focus on female gonads' development and maturation. Samples monthly collection was performed in collaboration with the local fishermen operating in the waters off the coast of the Marche region within a period of 1 year. The characterization of the ovary maturation stage, related to size class and time of the year, was performed to determine the actual sardines' reproductive cycle in the Adriatic Sea. Moreover, the histological analysis performed on the ovary revealed the presence of different structural anomalies such as the presence of indefinite structures in the ooplasm, anomalous coalescence of vitellogenin abnormal presence of previtellogenic oocytes, double oocyte structure-like, necrosis, blood vessels thickening of blood vessels walls and high concentration of white blood cells. The distribution of these abnormal features varies according to the age, size class and ovary maturation stage. Despite the economic and ecological importance of this species, this study represents the first comprehensive picture of the reproductive status of sardines in the Adriatic Sea. The present study also highlights the occurrence of several morphological anomalies indicating alterations in the oogenesis process and compromising the functionality of the gonad. New studies are needed to understand the cause of the onset of these anomalies

Keywords: sardine reproduction, oogenesis, ovary anomalies, maturation stage

SESSION 5:

GAMETE BIOLOGY

Chairman: Elsa Cabrita & Luz Pérez

[O22]

SPERM BEHAVIOR AND MOTILITY IN *ELASMOBRANCH* SPECIES: THE ROLE OF VISCOSITY

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Many *Elasmobranchii* species are considered endangered and may be extinct soon. As a part of *Chondrichthyans* (cartilaginous fishes), they belong to an old evolutionary class of vertebrates that diverged 450 mya. Modern cartilaginous fishes present a wide diversity of reproductive strategies while preserving the ancient mode of internal fertilization. The study of cartilaginous fish spermatology is important from evolutionary and general physiological perspectives as a part of reproduction studies. Moreover, understanding the principles of sperm motility is an essential step toward developing assisted reproductive techniques for this group of species.

Elasmobranchii species possess relatively big (compared to bony fishes) elongated spermatozoa with a spiral shape of the head, similar to one currently existing (but later diverged) in birds, reptiles, and amphibians. This specific spermatozoa shape can be considered an evolutionary ancient (plesiomorphic for vertebrates) type. These particular structures may be associated with the necessity to penetrate viscous ovarian fluid or the jelly layer of eggs, suggesting environmental viscosity as the driving force which shapes big-sized spermatozoa head to the spiral shape during evolution.

In the present study, we investigated the effect of surrounding media viscosity on spermatozoa motility parameters and their ability to propagate in three different *Elasmobranchii* species: the freshwater ray *Potamotrygon motoro*, the marine skate *Raja asterias* and the shark *Scyliorhinus canicula*. The spermatozoa of these species have slightly different spermatozoon head shapes, while all of them are still characterized by helical motion associated with the rotation around the longitude axes. This specific motion, when the head of spermatozoa screwing inside of media, is not efficient in low viscosity media, while in high viscosity results in a smooth and linear movement forward. Nevertheless, the head shape probably affects the velocity and other motility parameters in different species. Furthermore, surrounding media viscosity also helps spermatozoa release from spermatozeugmata more efficiently - 100% of spermatozoa are moved away due to their active motion. We also observed a specific (for this type of spermatozoa) motion, resulting in directional changes: transient backward motion and buckling of the head, both possible only in high viscosity media. Our experiments suggest that the viscosity of surrounding media could regulate spermatozoa motility and its performance during fertilization in the *Elasmobranchii* species.

Keywords: cartilaginous fishes, fish reproduction, spermatozeugmata

Acknowledgments: Supported by the projects: „CENAKVA“ (LM2018099) and “Biodiversity” (CZ.02.1.01./0.0/0.0/16_025/0007370 Reproductive and genetic procedures for preserving fish biodiversity and aquaculture).

[O23]

THE EFFECTIVENESS OF OVARIAN FLUID ON SPERMATOZOA PERFORMANCES IN MEDITERRANEAN BROWN TROUT WILD POPULATION (MOLISE REGION - ITALY)

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Knowledge of gamete quality is a prerequisite to fertilize eggs in an artificial environment, preserve gametes in gene banks, forecast recruitment variability for aquatic stocks, and understand natural mating processes. For externally fertilizing fishes, the ovarian fluid (OF) that surrounds the eggs is possibly a mediator of sperm selection. OF affects various sperm traits (sperm activation, chemotaxis, longevity, swimming performance and trajectory across) by modifying spermatozoa behaviours and fertilization outcomes, however, does not seem to affect sperm of all males in the same way, but varies depending on male and female identity, indicating cryptic female choice.

Here, we evaluated for the first time in Mediterranean brown trout (*Salmo cettii*) the effect of the OF on the sperm performance variability according to specific combinations of males and females. In particular, the study was conducted on a threated wild population of Mediterranean brown trout inhabiting the Biferno river (Molise region, Italy), where few breeding sites are reached by most of the migrating spawners resulting in high levels of reproductive competition.

Spermatozoa from eight males were activated in river water (RW) and OF from five females at 20% concentration. Sperm motility parameters were measured using computer-assisted sperm analysis (CASA). The mean of two independent activations was used for statistical analyses. Sperm motility parameters in RW and in OF were compared using two-tailed t-test. A generalized linear model (GLM) procedure was used to determine both the fixed effects of the male and female identity and their interaction on the sperm motility traits. In comparison to RW, OF significantly ($p < 0.05$) increased the sperm motility (85.1 *vs* 92.1%), sperm velocity parameters (curvilinear velocity [VCL], angular path velocity [VAP] and straight line velocity [VSL]) and prolonged the movement duration (23.1 *vs* 32 s). A significant male effect for all motility traits was found, whilst the female effect was significant only for VSL, straightness (STR), linearity (LIN), beat cross frequency (BCF) and duration of sperm movement. Interestingly, we found a significant interaction between male and female for all traits considered, indicating that OF from distinct females differently affected the sperm from specific males. In agreement with other studies, we can assume that trout spermatozoa with higher velocity and motility have the advantage of reaching the micropyle within a shorter time, which is crucial for fish that spawn in highly competitive environments. The male-female interactions observed in our *in vitro* study suggests that females could favour specific male genotypes or phenotypes, as a result of an evolutionary mechanism for sexual selection in an environment characterized by a high degree of reproductive competition.

Keywords: S. cettii, sperm traits, cryptic female choice, reproductive competition

Acknowledgments: Supported by the LIFE Nat.Sal.Mo. project (LIFE17 NAT/IT/000547).

[O24]

THE KICKER, THE HUGGER AND THE CATCHER: OVARIAN FLUID SPECIFICALLY DETERMINATES GAMETES ENCOUNTER IN RAINBOW TROUT, STERLET AND COMMON CARP IN LINE WITH THEIR REPRODUCTIVE BEHAVIOR

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Various fish species have versatile spawning behavior and tactics, which allows them to use sperm competition/selection effectively in the environmental conditions specific for different species. Ovarian fluid (OF) is likely an inevitable contributor to their reproductive tactics, affecting the gametes and their encounter.

The study compares the effect of ovarian fluid (OF) on fertilization performance (spermatozoa motility traits, chemotactic tests, *in vitro* fertilization) in three freshwater species which are taxonomically distant and differ by their reproductive behavior: rainbow trout, sterlet, and common carp. The reproductive behavior differences are well associated with OF basic features in these species (the volume, viscosity, content of ions), as well as the effects of OF on gametes' performance and *in vitro* fertilization. In particular, in the rheophilic rainbow trout, the females expel the eggs together with plenty of the OF into the water stream, and the male(s) ejaculated spermatozoa which enter this OF "cloud" start to move straightforwardly and significantly longer ("kicker" effect of the OF). The same ejaculate may include fractions of spermatozoa reacting differently, and most sperm cells stay in the area with optimal motility conditions. The *in vitro* fertilization test confirmed this positive effect of the OF on the procedure's outcome. In the sterlet, the females lay several batches of eggs covered by viscous OF, which protects them for a significant time against the effect of water; moreover, the OF temporarily inhibits spermatozoa motility and thus may "put on hold" spermatozoa from one or more males till the dilution of the ovarian fluid and motility re-activation ("hugger" effect). This was also confirmed by the *in vitro* fertilization. Common carp spermatozoa are extremely responsive to the OF presence, change the "conventional" linear mode of motility to an explorative one along the OF concentration gradient, and the OF, even at low concentrations, prolongs the activity period of spermatozoa: these features may represent an efficient strategy in case of free submerging egg coated by a thin viscous layer of the OF which occurs in the shallow waters ("catcher" effect). The presence of the OF had a significant positive effect on the *in vitro* fertilization outcome.

Thus, the environmental conditions that accompany the encounter of gametes, particularly the presence of ovarian fluid, significantly affect the performance of male gametes in rainbow trout, sterlet, and common carp, which is likely in line with their spawning behavior.

Keywords: freshwater fish, sperm motility, fertilization, maternal fluid, reproductive behavior

Acknowledgments: Supported by the Ministry of Education, Youth and Sports of the Czech Republic (project "CENAKVA ", LM2018099), and by the Czech Science Foundation (22-14069S).

[O25]

EFFECT OF LIGHT COLORS ON SPERMIATION AND SPERM KINETICS PARAMETERS DURING OUT OF SEASON REPRODUCTION IN POND-REARED EURASIAN PERCH (*PERCA FLUVIATILIS* L)

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Despite that commercial production of Eurasian perch has already been established in several countries, controlled reproductive protocols in this species still require further optimization, including lighting in hatchery condition. This is especially essential in Eurasian perch in which light spectrum has been reported to be important modulator of stress response. In the present study, we have focused on characteristic of pond-reared Eurasian perch sperm parameters following controlled wintering with application of different light colors (white, blue and red), hypothesizing that this might affect stress level and further reproductive capacity of males.

Pond-reared Eurasian perch males, obtained in early November were transferred to RAS and then were exposed to a 40 day-long wintering period before spawning. During entire experiment fish were exposed on three different light colors: white (W), blue (B) and red (R). 7 days before sperm collection, males from each lighting group were divided for two subgroups which one of them was stimulated for spermiation with 50 μg kg^{-1} of the sGnRHa. Semen was collected with a catheter from 5 males originated from each subgroup. The total volume of semen (VOL) was determined with accuracy of 0.1 ml. Sperm kinetics were examined with CASA system (SCA, Microptic S.L., Spain). Parameters which were chosen for analysis: MOT–percent of motile sperm (%), VCL–curvilinear velocity (μm s^{-1}), VAP–average path velocity (μm s^{-1}), VSL–straight line velocity (μm s^{-1}) and LIN-linearity of movement. All analyses were performed at a significance level of 0.05 using one-way ANOVA followed by HSD Tukey post hoc test.

No significant differences in spermiation success, semen volume and any CASA variables in response to light colors (white, blue or red) used during wintering period preceding out of season reproduction of Eurasian perch were found. Hormonal stimulation had a positive effect on total semen volume in all tested groups, however it had no effect on the observed sperm kinetics parameters, irrespective of light color. In conclusion, we consider that light color has no effect on spermiation and sperm kinetics parameters during controlled out of season reproduction in Eurasian perch creating possibilities for perch managers to freely adjust light colors. However, still the effect light color on stress and immune response indices should be further investigates to elucidate linkage between light color and physiological reaction in this species.

Keywords: light condition, wintering period, out of season spawning, CASA analysis

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[O26]

FIRST DESCRIPTION OF SUPEROXIDASE DISMUTASE MULTIGENE IN EUROPEAN EEL MALES: EXPRESSION *IN VIVO* UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Germ cells are specialized cells highly susceptible to be affected by oxidative stress through reactive oxygen species (ROS) attack. Among antioxidant enzymes, the superoxide dismutases (SODs) are the most important line of defense against ROS damage. In mammals, three SOD isoenzymes have been described: copper and zinc-containing SOD1; manganese-containing SOD2; and copper and zinc-containing SOD3 [1]. High levels of SODs are present in Japanese eel spermatogonia, and these are more tolerant to oxidative stress than advanced germ cells. Also, ROS inhibit androgen-induced germ cell proliferation, suggestingthat SOD activity loss affects the germ cell production. Without enough protection by SODs, germ cells and thus spermatogenesis may be jeopardized. Recently, transcriptome analysis of European eel males submitted to low temperature seawater revealed high expression of the three SODs in the testis, indicating that these genes play a physiological role in immature testis [2]. Our experiment consisted in applying cold water pre-treatments (during 2- or 4- weeks) to male European eels, followed by the standard hormonal treatment (salinity increase plus rechCG injections at 20 ºC for several weeks), in comparison with males treated directly with the standard treatment. We performed BLAST analyses in the European eel genomes and found one SOD1, two SOD2 and two SOD3, suggesting a duplication of SOD2 and SOD3 in this species. Then, we focused on describing the expression of SODs in different tissues and evaluated the pre-treatments and standard maturation treatment effects in these genes. SODs revealed tissue-specific expression, indicating that these have an important role in tissues from brain-pituitary-gonad axis, but also in non-reproductive ones. Salinity as well as hormonal treatment promoted the differential expression of all SODs. Cold seawater pretreatment before and with hormonal treatment applied at 20 ºC enhanced the expression of one SOD2 and the two SOD3 genes. These results were promising in terms of using low seawater as a pre-treatment, complementing the standard hormonal treatment, that can bea time-consuming and expensive process, while improving European eel spermiogenesis.

Keywords: Anguilla anguilla, spermatogenesis, spermatogonia

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References:

[1] Celino FT, et al. (2011). Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. *PLoS One*.

[2] Rozenfeld C, et al. (2019). Cold seawater induces early sexual developmental stages in the BPG axis of European eel males. *BMC Genomics.*

[O27]

PROTEOMIC ANALYSIS OF SIBERIAN STURGEON SEMINAL PLASMA THROUGH SHOTGUN AND GEL-BASED METHODS

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Sturgeons are recognized as “living fossils” since they are evolved around 200 million years ago. The biology and physiology of sturgeon reproduction differ substantially from teleost fish. Seminal plasma support and protect the viability, motility and fertilizing capacity of spermatozoa by creating the optimal environment for the storage. The aim of the study was in-depth proteomic analysis of Siberian sturgeon seminal plasma using gel based and gel-free proteomic approaches. Semen was collected from six mature males (weight 9±2 kg, age 7-9 years) maintained at the Department of Sturgeon Fish Breeding Inland Fisheries Institute in Pieczarki, Poland. For the first time, three different proteomic approaches were applied to characterize sturgeon seminal plasma proteins: LC-MS/MS, 2DE and 2D blue native (BN)/SDS-PAGE. LC-MS/MS allowed to identify 657 proteins, which were classified according to their functions and pathways. 2DE visualized 339 spots corresponding to 76 unique proteins (almost 60% were present in various proteoforms). For the first time we demonstrated interactions between seminal proteins; four (C1-C4) multiprotein complexes were identified after 2D BN/SDS-PAGE; composed of (C1) serotransferrin-2 (TF) and fish-egg lectin (FEL); (C2) serum albumin 2 (ALB) and retinol-binding protein 4 (RBP4); (C3) ALB and TF (C3) and (C4) apolipoprotein A-I (APOA1), riboflavin-binding protein (RfBP) and myoglobin (MB). BN-SDS-PAGE also revealed that immunoglobulin and TF formed homomultimeric (dimer, trimer, tetramer and pentamer) complexes in seminal plasma. ALB, TF, Beta-Ala-His dipeptidase, glyceraldehyde-3-phosphate dehydrogenase, IGH, APOA1, hemopexin, type-4 ice-structuring protein, FEL, RfBP and glycogen phosphorylase, liver form-like were selected as major seminal plasma proteins. The functional analysis of sturgeon seminal plasma proteins indicated their involvement in immune system process and response to stimulus, metabolism, vesicle-mediated transport, proteolysis, and catherin binding involved in cell-cell adhesion. Moreover, 67 proteins were associated with reproductive processes, including spermatogenesis, fertilization, acrosomal reaction and sperm motility. Comparative proteomic analysis of sturgeon seminal plasma with other fish species and human showed common proteins with fish and human and 150 proteins specific for sturgeon which may reflect the specificity of sturgeon reproduction. To conclude, this work represents the first in-depth proteomic characterization of sturgeon seminal plasma proteins and their complexes. This integrative view lead to deeper insight into physiological function of seminal plasma and processes occurred in sturgeon reproductive tract.

Keywords: Acipenser baerii, fish, proteome, mass spectrometry, electrophoresis

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PLENARY KEYNOTE

[O28]

EPIGENETICS IN FISH GAMETES: SENSITIVITY TO ENVIRONMENTAL CLUES AND TRANSGENERATIONAL ACCLIMATION

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Epigenetic landscapes define cell identities by regulating chromatin 3D architecture and transcriptional programs. They rely on DNA methylation and histone post-translational modifications, which are both transmittable through many cell divisions and even generations, and reversible upon physiological or environmental context. A key issue in developmental biology involves how the embryos inherit and assimilate epigenetic information from parental gametes in order to restore pluripotency and allow the development of a new individual. In addition, epigenetic marks being the molecular interface between the environment and the genome, the study of their sensitivity to various stresses is of particular interest in gametes, where alterations might influence the phenotypical performances of the next generation(s). Recent advances in the field of parental epigenetic contribution to embryo development will be presented, focusing on fish and male gametes. This knowledge is instrumental to address numerous fish biology or aquaculture related issues, from the comprehension of transgenerational acclimation to environmental clues to the prediction of phenotypes or the assay of sperm quality.

Keywords: DNA methylation, histone post-translational modifications, development, embryo

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SESSION 6:

GAMETE STORAGE AND CRYOPRESERVATION

(Part I)

Chairman: Ákos Horváth & Sylwia Judycka

[O29]

CRYOPRESERVATION AFFECTS CELLULAR QUALITY BUT NOT DNA METHYLATION PROFILE IN RAINBOW TROUT SPERMATOZOA

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Sperm cryopreservation is a widely used biotechnology in fish. In the spermatozoa, DNA bears the genotype of the donor male, whose transmission to the progeny will allow the restoration of valuable genetic resources. Additionally, it is now known that fish spermatozoa epigenetic profile, particularly DNA methylation, is transmitted to the embryo. As a consequence, any alteration of DNA methylation in spermatozoa induces the risk of transmitting epigenetic alterations to the offspring leading to altering the progeny. Published data on cryopreservation impact on DNA methylation are limited and contradictory because they are species and cryoprotectant dependent. The aim of this study is to assess whether cryopreservation of rainbow trout spermatozoa can alter their DNA methylation profile and whether the thawed sperm cellular quality is related to DNA methylation alterations. We also wanted to investigate whether the cryoprotectant molecules play a role in DNA methylation alterations. Sperm from rainbow trout mature males (n=12) was cryopreserved with dimethylsulfoxide (DMSO), methanol (MeOH) or glycerol and compared to fresh sperm. Thawed spermatozoa quality was assessed from their plasma membrane integrity and fertilization ability. DNA methylation was studied by Reduced Representation Bisulfite Sequencing (RRBS). Our results showed that membrane integrity is best preserved with DMSO and very well preserved with glycerol whereas methanol provided a lower membrane integrity protection. Fertilization ability data confirmed that DMSO is the best cryoprotectant and that methanol spermatozoa protection is variable. However, glycerol did not preserve thawed spermatozoa fertilization ability. Despite the variability in thawed spermatozoa quality depending on the cryoprotectant, our data demonstrated that cryopreservation did not alter DNA methylation of cryopreserved spermatozoa regardless of the cryoprotectant used. This result was observed at the global scale and confirmed at the base resolution. To conclude, DNA methylation is a robust epigenetic actor unaffected by cryopreservation regardless of the cryoprotectant used. This reveals that fish farming of rainbow trout can safely use sperm cryopreservation.

Keywords: *biotechnology, epigenetics, gamete, fish*

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[O30]

SPERM FRACTIONATION IN FISHES: DENSITY GRADIENT CENTRIFUGATION AND MICROFLUIDIC SEPARATION APPROACHES FOR CRYOPRESERVED SAMPLES

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There are some pieces of evidence that the separation of motile fractions from sperm can increase the likelihood of successful insemination, which is essential for medicine and animal breeding. As the freeze-thawing process leads to decreased sperm motility parameters, the application of separation can be considered a useful tool to increase insemination outcomes and to understand real cryopreservation effects on spermatozoa. The latter is usually obscured by the presence of sperm fractions differentially cryodamaged in the same samples. Nevertheless, the methods of sperm separation were not intensively tested in fish, in which spermatozoa become motile for an extremely brief period. That property and small fish spermatozoon dimensions complicate the separation of motile sperm from the whole population. The current study is aimed to compare cell microfluidic acoustic separation and Percoll density gradient centrifugation approaches for obtaining the motile fractions of sperm from cryopreserved samples in fishes possessing taxa-specifically different spermatozoa.

The study used samples of common carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss*, sterlet *Acipenser ruthenus* and Siberian sturgeon *A. baerii* frozen and thawed by routine methods. After applying separation, obtained fractions of spermatozoa were analysed by CASA to evaluate sperm motility percentage and velocities. Among tested motility parameters, sperm motility percentage was the most affected by microfluidic acoustic separation since other CASA parameters were not significantly different between fractions. Sperm motility percentage in separated fractions was significantly higher in trout, Siberian sturgeon and sterlet but not in common carp. However, Percoll density gradient centrifugation in sterlet and carp separated spermatozoa with motility percentage and velocities statistically higher than values for not separated samples. It was suggested that these two methods of separation based on different physical properties of cells might result in a separation of different fractions of motile spermatozoa in a taxon-specific way. Further, a more detailed study and comparison of the efficiency of sperm separation methods are required. It is the first report on applying cell microfluidic acoustic separation techniques for fish sperm.

Keywords: Percoll, acoustic cell separation, CASA, motility parameters

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[O31]

EFFECT OF SHORT-TERM STORAGE SPERM IN COMMON CARP (*CYPRINUS CARPIO*) ON SPERM DNA METHYLATION AND EPIGENETIC INHERITANCE LEVEL IN EMBRYOS

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Short-term storage of sperm *in vivo* and *in vitro* is a practical technique in the artificial fertilization of fish, which can cause changes in DNA methylation because of sperm aging, with the risk of transmission to the embryos. The purpose of this study in common carp was i) to analyze phenotypic characteristics of aging sperm, ii) to investigate whether sperm DNA methylation is affected by aging and iii) to explore whether changes in sperm DNA methylome caused by aging may be transferred to the embryos, with deleterious effects. After multiple hormonal stimulations of the males, sperm was stored *in vivo* for up to 3 days, then collected and stored with extenders *in vitro* for up to 6 days. Embryos at early- and mid-blastula stages were obtained from fresh, *in vivo* and *in vitro* aged sperm groups. Spermatozoa phenotypes were analyzed using CASA. Global DNA methylation/hydroxymethylation level and gene-specific DNA methylation in fresh and aged spermatozoa and their embryos were investigated using LC-MS/MS and whole-genome bisulfite sequencing (WGBS), respectively. The results demonstrated that spermatozoa aging of common carp significantly affects sperm performance and artificial fertilization. The methylation level at the CpG sites increased significantly with spermatozoa stored *in vitro* without an extender for 1 day compared to the fresh group and then decreased significantly at 4 days. No significant differences were found in global DNA methylation/hydroxymethylation of the embryos sired with fresh and sperm aged *in vitro* with extenders or stored *in vivo*. Likewise, although few differentially methylated cytosines were found between the carp embryos sired with fresh and aged sperm, they accounted for less than 1/500 000 of the carp genomic CpGs. These data would indicate that the offspring did not carry on the methylation changes observed in sperm. Our results provide clues for future studies on the epigenetic mechanisms involved in sperm aging.

Keywords: sperm quality, epigenetics, fertilization, embryos, sperm aging, WGBS

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[O32]

CRYOPRESERVATION OF THE TESTICULAR TISSUE AND XENOTRANSPLANTATION OF EUROPEAN EEL (*ANGUILLA ANGUILLA*) SPERMATOGONIA

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The European eel is a valuable product in the markets. Nowadays it is possible to obtain eel gametes after long and expensive hormonal treatments, but there is often high variability in their quality. In recent decades, the development of breeding techniques, including surrogacy technology, has been a major focus of study. Our objectives were *i*) to develop methods to preserve European eel spermatogonial stem cells (SSCs) *ii*) to test the functionality of SSCs isolated from cryopreserved testis by xenotransplantation into common carp (*Cyprinus carpio*), a species that has perfectly controlled its reproduction in captivity.

Immature eel testes were dissected to develop and optimize the protocol of SSCs cryopreservation (slow-rate freezing, vitrification, and hypothermic storage). For freezing, 6 cryoprotectants at different concentrations, as well as sugar and protein supplementation were tested. The optimized cryomedium contained 1.5 M of dimethyl sulfoxide (Me_2SO) with 0.1 M glucose and 1.5% BSA with the highest average viability reaching ~50%. For vitrification, 3 different equilibration, vitrification, and warming solutions were tested. The highest average viability of ~70% was obtained by using propylene glycol and Me_2SO in equilibration and vitrification media. Hypothermic storage at 4 °C in L-15 medium was better in cell suspensions than in tissue pieces, with 75% viability after 144 h. To test the functionality of the cells, SSCs from thawed testis were stained using PKH-26 dye and microinjected into sterilized carp larvae. After 1.5 months, fluorescently-labelled cells were observed in the gonads of 8 out of 25 carps.

In conclusion, the technique to cryopreserve viable European eel SSCs has been developed. Monitoring to determine the fate of the transplanted cells will demonstrate if the phylogenetically distant common carp could be a possible surrogate recipient species for the European eel.

Keywords: testis cryopreservation, common carp, germ cells, transplantation, spermatogonia

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[O33]

NEXT STEPS ON THE APPLICATION OF TECHNIQUES FOR THE CONTROL OF REPRODUCTION OF CHONDRICHTHYAN SPECIES

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Chondrichthyans (informally referred as sharks, rays, and chimaeras) are considered one of the most threatened groups of vertebrates. Given this situation, *ex situ* breeding programs could be considered as a strategy on the conservation of certain species. However, these breeding programmes require the control of the reproduction of the animals to be successful. Therefore, some assisted reproductive techniques, such as sperm collection, management, evaluation, and preservation, need to be further developed.

Using the small spotted catshark *Scyliorhinus canicula* and the rough skate *Raja radula*, as model species, we have been able to develop techniques to obtain viable sperm in up to 19 different chondrichthyan species. Sperm extraction has been performed after a detailed description of the reproductive anatomy of each species, due to the anatomical diversity between them. The sperm has been obtained through cannulation, abdominal massage, and dissection, in both live and dead animals. Viable sperm was also obtained in females via dissection of their oviducal glands.

To work with this sperm, we have formulated an artificial seminal plasma that can be used to maintain spermatozoa motility for >30 days at 4 °C. After supplementing this formulation with different combinations of cryoprotectants, i.e., methanol, dimethyl sulfoxide (DMSO), and fresh egg yolk, we were able to successfully cryopreserve the sperm of 15 chondrichthyan species. Samples were frozen inside a Styrofoam box using vapor of liquid nitrogen and preserved into liquid nitrogen. Pre-freezing and post-thawing sperm quality was assessed and compared by studying the sperm cells motility and their membrane integrity. In rays, the use of 10% DMSO or 10% methanol rendered motility values higher than 40%. In sharks and chimaeras, the best motility values were obtained combining 5% DMSO, 5% methanol and 10% egg yolk, which induced post-thawing motility values close to 35%. The information obtained from the species studied expand our knowledge on the use of reproductive techniques applicable to chondrichthyans. It also lays the groundwork for the first sperm cryobank for these or similar species.

Keywords: cryopreservation, captive breeding, cryobanking, ex situ conservation, elasmobranchs

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[O34]

PROTOCOL OPTIMIZATION FOR SEA BASS SPERM CRYOPRESERVATION AND ASSESSMENT OF POST-THAWING DILUTION TO PROLONG SPERM USEFULNESS IN AQUACULTURE MEDITERRANEAN SPECIES

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Our study aimed to optimize the sperm cryopreservation protocol of sea bass (*Dicentrarchus labrax*) and evaluate the post-thawing sperm dilution in species of interest for Mediterranean aquaculture. Sea bass sperm samples (n=6) were cryopreserved using a modified Non-Activating Media (NAM) extender with or without 5% Glucose (Glu), and 10% of Me_2SO or MeOH as cryoprotectant. In a second experiment, sea bass sperm samples (n=7) were cryopreserved using a modified NAM extender and Me_2SO. In both experiments, an aliquot of post-thawed sperm was diluted in NAM or NAM + Bovine Serum Albumin (BSA), or not diluted (control). Senegalese sole (*Solea senegalensis*) sperm samples (n=13) were cryopreserved. The post-thawed sperm was diluted in Mounib or NAM media, or not diluted (control). European eel (*Anguilla anguilla*) sperm samples (n=12) were cryopreserved. An aliquot of post-thaw sperm was diluted in P1 or P1 + BSA media, or not diluted (control).

Kinetic parameters were checked after thawing: sea bass (0, 2.5 and 6 h), Senegalese sole (0 and 3 h), and European eel (0, 24, 48, 72 and 96 h). The total motility (MOT - %), progressive motility (MOTp - %), the velocities curvilinear (VCL - µm/s), straight line (VSL - µm/s) and average path (VAP - µm/s), were evaluated using a CASA-Mot software. The highest post-thawing kinetic parameters of sea bass sperm were observed when Me_2SO or Me_2SO+Glu were used and post-thawed sperm was diluted in NAM+BSA. The post-thaw sperm dilution increased kinetic parameters and prolonged sperm capacity compared to undiluted (control) samples. The post-thawing dilution did not increase the quality of the Senegalese sole sperm at 0 h. However, after 3 h, the spermatozoa diluted in NAM maintained the VCL. The European eel post-thaw sperm diluted in P1 maintained the MOT at 48 h and the velocities at 96 h after thawing.

The combination of Me_2SO+Glu can be used to cryopreserve sea bass sperm, and MeOH should be avoided. Sea bass post-thawing sperm dilution increases sperm kinetic parameters and prolongs its use at 2.5 h. Senegalese sole post-thaw sperm dilution did not affect the sperm kinetic parameters. European eel post-thaw diluted in NAM can be used without losing quality for 48 h after thawing.

Keywords: Dicentrarchus labrax, Solea senegalensis, Anguilla anguilla, aquaculture

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[O35]

EFFECT OF AN ANTIBIOTIC-FREE MEDIUM ON MILT MOTILITY AND FERTILITY OF RAINBOW TROUT SEMEN AFTER MID-TERM STORAGE AT 4°C

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Antibiotic resistance is identified, by WHO (World Health Organization), as one of the top 10 big threats to global health. In daily farming of salmonids production, tremendous progress has been made in grow-out production stages to develop effective and efficient alternatives to antibiotics. Thus, the removal of antibiotics from semen dilution media, such as Storfish medium (Ref. 018500, IMV Technologies, France), is a priority. This present study aimed to study the effect of an antibiotic-free Storfish on rainbow trout (*Oncorhynchus mykiss*) semen quality and fertility for a mid-term liquid storage (22 days) at 4°C.

Gonads were collected from mature rainbow trout sex-reversed females (n = 11). Gonads were then cut into small fragments, filtered and the recovered semen samples were diluted 1:2 (w:v) in four media: three industrialized batches of antibiotic-free Storfish (AB-free Storfish) and Storfish with antibiotics (control). Diluted semen samples were then packaged in GTB bag (IMV Technologies, L'Aigle, France), fully filled with pure oxygen (5 ml diluted milt filled in 80 ml GTB bag) and kept for 22 days at 4°C. Sperm motility and velocities were measured at D0, D1, D3, D8, D13, D17 and D22 with a CASA system (IVOS II, IMV Technologies, France). A microbiological count was performed at D2 and D13. Fertilizations were performed on D1, D8 and D22 with three sperm-to-egg ratios (i.e., 500,000:1, 50,000:1 and 10,000:1). Results were analyzed using linear mixed models on R software.

After microbiological analysis, no bacterium was found at D2. However, at D13, contamination was observed in some samples from AB-free and control Storfish and could be counted without dilution. The bacteria identified were from *Pseudomonas fluorescens* group. Semen motility was shown relatively stable until D3 and decreased significantly from D8 to D22 compared to D1 for all media. Despite a decrease in total motility over time, velocities were at the highest at D3 and remained stable over time. No significant difference in motility nor velocities was observed between AB-free and control Storfish. After fertilization, the highest fertility rate (95 %) was observed at D8 with 500,000 sperm cells/egg and the lowest (11 %) at D22 with 50,000 sperm cells/egg (all media combined). A significant effect of the time and the sperm-to-egg ratio was observed. However, no difference was found between media at D8 and D22.

To conclude, no significant negative effect of antibiotic-free Storfish was observed compared to Storfish with antibiotics during the whole storage period. Thus, the validation of the antibiotic-free Storfish will help to address some of the concerns about antibiotic resistance in the future for aquaculture production.

Keywords: Salmonids, antibiotics, liquid preservation, storfish, milt motility, fertilization

SESSION 6:

GAMETE STORAGE AND CRYOPRESERVATION

(Part II)

Chairman: Catherine Labbé & Victor Gallego

[O36]

REPLACEMENT OF MATERNAL GERM PLASM IN STURGEON

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Sturgeon is one of the most critically endangered species in the world mainly due to overfishing for caviar, it is urgent and necessary to establish the new technique to reproduce sturgeon. The cryopreservation of sperm has been successful in several aquatic species, but we still lack the technology to cryopreserve eggs and embryos. The reasons include big size of oocyte, high yolk content andlow permeation to cryomedium. Not being able to conserve the maternal genetics limits the gene pool and conservation efforts. Maternal genetics meaning mitochondria, mtDNA can be easily cryopreserved.

The aim of the present research is to implement the interspecific mitochondrial transplantation technique to sturgeon. Mitochondria were isolatedfromeggs of endangered sturgeon, then host embryos would undergo irradiation of vegetal pole with UV to eliminate the endogenous germplasm, and the previously isolated mitochondria would be injected into the vegetal pole of the embryo at the 1-4 cell stageallowing the mitochondria to colonize primordial germ cells. The importance of this first attempt to apply mitochondrial transplantation in sturgeon species is a first step towards achieving interspecific replacement of mitochondria in sturgeon germline. This technique could have wide reached impacts on endangered fish species where oocyte freezing is not possible and provide with great hope in sturgeon's conservation.

Keywords: mitochondria, transplantation, maternal germ plasm, replacement

[O37]

EMBRYOS PRODUCED FROM POST-OVULATORY AGED OOCYTES IN COMMON CARP (*CYPRINUS CARPIO*) EXHIBIT ABERRANT EPIGENETIC MODIFICATIONS OF GLOBAL DNA HYDROXYMETHYLATION

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Fish embryos originated from post-ovulatory aged oocytes experience delayed developmental competence and exhibit increased ploidy anomalies and phenotypic malformations. The underlying molecular mechanisms linking post-ovulatory oocyte ageing with the subsequent deleterious effects on the embryos are still insufficiently understood. Aberrant epigenetic modifications are a hallmark of ageing process. An important epigenetic mechanism is the coordinated methylation or demethylation of CpG islands in the promoter region of specific genes. In the present study, common carp (*Cyprinus carpio*) oocytes were aged for 12 h *in vitro* at 20 °C and their capacity to fertilize and progress to the larval stage were evaluated. Genome-wide DNA (hydroxy) methylation was analyzed in 30 h embryos developed from fresh and aged oocytes using liquid chromatography tandem-mass spectrometry (LC-MS/MS) method. Samples of genomic DNA (10 µg) were hydrolyzed to 2´-deoxynucleosides. [$^{15}N_2$,$^{13}C_1$]dC, 5-mdC-d_3 and 5-hmdC-d_3 were used as internal standards for quantification of dC, 5-mdC and 5-hmdC in DNA hydrolyzates. The following mass transitions were used for quantification (accordant internal standard in parentheses): dC: *m/z* 228 (231) → 112 (115); 5-mdC: *m/z* 242 (245) → 126 (129) and 5-hmdC: *m/z* 258 (261) → 142 (145). Results of the present study demonstrate that genome-wide DNA hydroxymethylation decreased in embryos produced from more advanced aged oocytes. The 5-hmdC/dC levels were 30% lower in embryos originated from 12 h aged oocytes, as compared to those produced from freshly ovulated oocytes. The genome-wide DNA methylation was not affected by oocyte ageing. 5-mdC/dC levels were similar in embryos produced from fresh and aged oocytes (approximately 10% 5-mdC/dC). This study is the first to investigate genome-wide DNA (hydroxy) methylation in embryos arised from different aged oocytes and indicates that epigenetic modifications of DNA methylation might be involved in underlying mechanisms of embryo failures as sociated with post-ovulatory oocyte aging. Our research group is currently applying the whole genome bisulfite sequencing to detect differentially methylated regions and thereby to provide a more detailed investigation of gene specific differences in the DNA methylation.

Keywords: embryo, fertilization, genome-wide DNA (hydroxy)methylation, oocyte ageing

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[O38]

HISTONE MODIFICATIONS DURING OOCYTE AGEING IN COMMON CARP (*CYPRINUS CARPIO*)

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Oocyte quality and the arising progeny's healthiness can be negatively influenced by post-ovulatory oocyte ageing. The possible role of epigenetics in cellular ageing has been depicted. Post-translational modifications on histone proteins are among the most crucial and common epigenetic regulators that play a critical role in the success of early embryo development. The current study investigated the global histone modifications during *in vivo* and *in vitro* post-ovulatory oocyte ageing in common carp *Cyprinus carpio*. In addition, the key sites of acetylation on lysines K9 and K14 in histone H3 and lysines K5, K8, K12, and K16 in histone H4 were investigated. Histone acetyltransferase activity was also assessed to obtain more evidence on the dynamics of histone acetylation during oocyte ageing. The global histone modifications were not altered for up to 8 hours of *in vivo* and *in vitro* oocyte ageing. Examining the specific histone acetylations revealed the presence of H3K9ac, H4K5ac, H4K8ac, and H4K12ac, as well as the absence of H3K14ac and H4K16ac in common carp metaphase II oocytes. Acetylation on H3K9, H4K5 and H4K8 did not exhibit significant differences in different aged oocytes. No signal was detected for the histone H3K14ac and H4K16ac in either fresh or aged oocytes. A significant hyperacetylation was observed on H4K12 after 28 hours of *in vitro* oocyte storage. Although not significant, an increasing trend of histone acetyltransferase activity was observed during both *in vivo* and *in vitro* oocyte ageing. In conclusion, histone acetylation as a crucial epigenetic mediator may be associated with age-related defects, particularly in oocytes of more advanced age. Identifying the genomic regions associated with the altered histone modifications due to oocyte ageing is of our interest for the upcoming studies.

Keywords: egg quality, epigenetics, histone acetylation, histone acetyltransferase, post-ovulatory ageing

Acknowledgments: Supported by the Ministry of Education, Youth and Sports of the Czech Republic, project: LRI CENAKVA LM2018099, and by the Czech Science Foundation (GACR No. 20-01251S).

[O39]

INVESTIGATION OF INHERITED CRYORESISTANCE IN ZEBRAFISH AND COMMON CARP

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A 4-year multi-generation study was performed in the zebrafish (*Danio rerio*) and the common carp (*Cyprinus carpio*) in order to investigate if the sperm of individuals hatched from eggs fertilized with cryopreserved sperm shows greater tolerance towards cryopreservation than that of individual hatched from eggs fertilized with fresh sperm. This phenomenon was first observed in the rainbow trout by Babiak et al. [1] suggesting epigenetic alterations during the process of freezing and thawing.

In the zebrafish 8 full-sib families were created and grown until generation F_3 using the cryopreserved and fresh sperm of selected males. Similarly, 6 fulls-sib families were created and grown until generation F_2 in the common carp using the same experimental setup. In each subsequent generation, sperm samples were collected, their fresh as well as post-thaw motility, concentration as well as fertilizing capacity was determined. Additionally, in the common carp, landmark-based geometric morphometric methods were used to test alterations in the body shape of various groups.

Due to the low volumes of sperm collected in zebrafish individuals, no statistically significant differences were found between the sperm quality parameters of fish originating from fresh or cryopreserved sperm in any of the investigated generations (F_1, F_2 and F_3). In the common carp, motility tests of males in the F_1 generation have shown that the family of fish had no significant effect, while both the sampling date ($P < 0.001$) and the origin of males had a significant effect ($P = 0.024$, $N = 46$ for cryopreserved, $N = 63$ for fresh) on the progressive motility of cryopreserved carp sperm. No significant difference ($P = 0.86$) was found between the fertilizing capacity of cryopreserved (87 ± 5%) and fresh sperm (86 ± 13%) of F_1 males used to establish the F_2 generation. Canonical variate analysis (CVA) of common carps individuals in the F_2 generation revealed that fish originating from cryopreserved sperm generally had a significantly smaller head, lower back and narrower caudal peduncle than those originating from fresh sperm.

Keywords: zebrafish, carp, sperm, cryopreservation, morphometry

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References:

[1] Babiak I, et. al. (2002). Semen from rainbow trout produced using cryopreserved spermatozoa is more suitable for cryopreservation. *J Fish Biol.*

[O40]

TRANSCRIPTOMIC CHARACTERIZATION OF C*RASSOSTREA ANGULATA* CRYOPRESERVED D-LARVAE

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The Portuguese oyster *Crassostrea angulata* is a bivalve of major economic value and widely distributed around the world. Nowadays not only its production but also its natural populations are endangered due to climate and anthropogenic factors. To overcome this situation there is a need to create tools and strategies to preserve this species natural banks and to develop aquaculture. Cryopreservation is a valuable candidate to secure the storage of genetic lines of endangered species, preserving biodiversity, and helping in the management of reproduction. However, cryopreservation procedures may induce different types of damage, leading to a depletion in the quality of post-thaw larvae. The use of next-generation sequencing techniques such as RNA-seq may create new insight on the molecular consequences of cryopreservation, allowing the identification of biological markers to assess larval quality. The aim of this study was to evaluate the quality of *C. angulata* D-Larvae after cryopreservation. With this objective, swimming activity, morphology, and a whole transcriptome analysis were performed. D-larvae pools of 60000 D-larvae/ml were obtained through artificial fertilization and divided into 9 aliquots. Three aliquots of fresh larvae were used as control, three were exposed to the cryoprotectant solution, and the last three were cryopreserved in a programmable biofreezer using 0.5ml straws. The cryoprotectant solution used in both treatments was composed by 20% (v/v) DMSO, 2% (w/v) polyvinylpyrrolidone 40000 MW and 0.4 M sucrose prepared in milli-Q water. The total RNA of each pool was extracted and sequenced following a standard RNA-seq workflow. Differential gene expression and gene set enrichment analysis were performed and results were confirmed through real time quantitative PCR. The selected genes were previously reported to be related to structural and functional damage in bivalves. No significant differences on swimming activity in terms of motility and velocity parameters was found. In contrast, morphological alterations in larvae exposed to the cryoprotectant solution were significantly higher. Expression analysis identified only 3 differentially expressed genes (DEG) between the control and the cryoprotectant exposure treatment. However, between the control and the post-thaw treatment, 611 DEG's were identified. This data suggests that D-larvae quality was mostly affected by the freezing process in comparison to any possible cryoprotectant toxic effect. The set of genes selected for real time quantitative PCR corroborated the RNA-seq results and were considered to be possible biomarkers of freezability for D-larvae quality assessment.

Keywords: cryopreservation, oyster, RNA-seq, dimethyl sulfoxide, cryodamage

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POSTER SESSIONS

[P1]

APPLICATION OF UV-IRRADIATED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) SPERMATOZOA TO INDUCE GYNOGENETIC DEVELOPMENT OF THE EUROPEAN GRAYLING (*THYMALLUS THYMALLUS*)

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Production of all-female grayling (*Thymallus thymallus*) stocks might be of a future interest to the aquaculture sector for the production of all-female triploids. In salmonids, all-female triploids are sterile and so can be used as safe stocking material, excluding risk of the detrimental genetic introgression to the native populations. In fish with XX/XY sex determination system, production of all-female offspring within one generation may be achieved by the artificial gynogenesis. Thus, the main goal of our research was to provide gynogenetic progenies of the European grayling. Successful induction of the gynogenesis has been achieved by activation of eggs with the UV-irradiated rainbow trout sperm and exposure of the inseminated eggs to the high hydrostatic pressure (HHP) shock (9,000 psi for 5 min) 20 min. after activation performed at 10^{o} C. The survival rates of the gynogenetic larvae exhibited huge inter-clutch variability and ranged from 0.96 ± 0.33% to 60.57 ± 1.85%. The application of microsatellite DNA analysis confirmed only maternal inheritance in the grayling larvae that hatched from eggs inseminated by inactivated heterologous sperm. All examined gynogenotes were confirmed to be genetically females. Ratio of deformed larvae that hatched from eggs activated by UV-irradiated heterologous milt reached up to 37.27 ± 10.08%. Despite increased ratio of fish with body deformities, gynogenesis of the European grayling achieved by activation of eggs with UV-irradiated rainbow trout sperm followed by HHP shock proved to be reliable and fast method for production of all-female grayling stocks.

Keywords: all-female, European grayling, gynogenesis, heterologous spermatozoa, pressure shock, UV-irradiation

[P2]

EVALUATION OF THE EFFICACY OF ACETON-DRIED COMMON CARP PITUITARY DURING INDUCED BREEDING OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AFTER AN EXTREMELY LONG-TERM STORAGE

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Hypophysation is the oldest and most widely used method of induced fish breeding. In this technique, crude extracts of piscine pituitary gland are homogenized and injected into the recipient fish. In practice, common carp (*Cyprinus carpio*) pituitary is one of the most commonly used agents to induce ovulation. The active ingredients of pituitary (gonadotropins) are heterodimeric glycoproteins including follicle stimulating hormone and luteinizing hormone. Since they are very susceptible to fast degradation, the glands must be preserved immediately after collection. The dissected glands are put in acetone for defatting and dehydration. After 24 hours they are placed on a filter paper and allowed to dry at room temperature. Acetone-dried glands are stored whole at room temperature until required. According to the literature, they can be preserved for at least 4–6 years if they are kept free from moisture.

The aim of our experiments was to investigate the efficacy of an extremely long-stored batch of pituitary gland collected in 1967. The biological potential of the glands was assessed through reproductive indices recorded during induced breeding of African catfish (*Clarias gariepinus*). The experiments were performed in October 2020. Two groups of African catfish females containing 10 females each were placed in separate tanks. One group was treated with acetone-dried carp pituitary collected in 1967 and the other (control) group was injected with pituitary collected in the year prior to the experiments. After ovulation eggs from each female were fertilized with the same pool of sperm collected from several males. To assess the effectiveness of pituitary treatment the following reproductive traits were determined: ratio of ovulated females, relative weight of the stripped eggs and the residual ovary, fertilization and hatching rates. All treated females ovulated in both groups. The response of females to the treatments met the expectations for all reproductive traits. The statistical methods used did not indicate a significant difference between the two groups for any of the reproductive indices, which means that the pituitary gland collected in 1967 remained effective after 53 years.

Keywords: long-term storage of carp pituitary, induced breeding of African catfish

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[P3]

EFFECTS OF HUMAN-ANIMAL INTERACTION DURING SENEGALESE SOLE CULTURE ON STRESS, GROWTH AND SEX STEROID HORMONE LEVELS

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Solea senegalensis is a flatfish of high commercial value in Europe. An adequate biomass control in fish farming is crucial to guarantee optimal growth. Periodical protocols for biomass calculation require human handling and can cause stress on the individuals, having a potential negative impact on growth [1], reproduction, immune system and welfare. This work focuses on determining the effects of reduced levels of human-animal interaction on the Senegalese sole culture in terms of cortisol and sex steroids levels in plasma and growth.

All animals were manipulated according to the Guidelines of the European Union Council (2010/63/EU), following Spanish regulations (RD/2013) for the use of laboratory animals. 90 adult Senegalese soles born and reared in captivity were split into two groups and distributed within three tanks replicates (n=15 per tank) to reach approximately the same initial biomass. The control group was standard manipulated, which included periodical (monthly) human-animal interaction for measuring weight and length whereas the experimental group was maintained under lower human handling. After 6 months a biometry analysis was performed and blood samples from control and experimental individuals were extracted. Plasma levels of cortisol (indicator of stress response), testosterone and 11-ketotestosterone were determined (n=7-9) using commercially available enzyme immunoassay kits (Cayman, USA).

Results regarding specific growth rates (SGR, % d) (month 1 to 6) of control and experimental groups did not report differences. However, the group of reduced human-animal interaction showed a clear tendency (p=0.056) to have greater values of SGR (0.46±0.012 % d) when compared to standard human-handled animals (0.43±0.007 % d). In terms of stress effects, cortisol plasma levels were higher (p=0.017) in the control (standard manipulated) group (54.3±17.7 ng/ml) than in the experimental group (lower human-animal interaction) (5.2±1.4 ng/ml), showing the stressful effect of human handling. Plasma testosterone concentrations were higher, but non-significant, in less manipulated animals (p=0.086) and plasma 11-ketotestosterone concentrations did not differ between the two animal groups. Further studies will be focused on analyzing the effect of lower human interaction in adult fish during longer periods of time as well as on analyzing its effect in juveniles.

Keywords: Senegalese sole, stress, steroid hormones, cortisol, growth

Acknowledgments: Supported by PID2019-108509RB-I00 project (Ministerio de Ciencia e Innovación), FCJ2018-037566-I grant and STOLT Sea Farm.

References:

[1] Martínez-Vázquez JM, et al. (2019). Artificial Neural Network (ANN) as a Tool to Reduce Human-Animal Interaction Improves Senegalese Sole Production. *Biomolecules*.

[P4]

A FEASIBLE METHOD FOR SENEGALESE SOLE SPERMATOGONIA ENRICHMENT

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Spermatogonia are increasingly used in biotechnological innovation related to transplantation in endangered or commercially cultured fish species. However, in some species spermatogonia enrichment is a crucial step in the success for further transplantations or cryopreservation techniques. The aim of this study was to develop an efficient protocol for the enrichment of Senegalese sole spermatogonia. Senegalese sole (*Solea senegalensis*) is a flatfish species farmed in the South of Europe with high market price, but that presents a reproductive disorder which does not allow to close the live cycle in captivity, making this species propitious to spermatogonial biotechnological applications. In this perspective, 4 pools containing testes from 8 juvenile fish (~1-year-old and 35-50 g of weight) were prepared for spermatogonia enrichment using plates coated with different molecules, e.g. laminin, collagen and gelatine, and strainers of different pore size (from 1 to 30 µm). Testes were cut in 1 mm^3 fragments and dissociated using mechanical and enzymatic treatment and then passed through a 100 µm-strainer. The effectiveness of the different enrichment strategies was assessed at each step through the calculation of the percentage of spermatogonia and expression of genes specific of spermatogonia (*gfra, pou5f/oct4, notch1, mcm6, ly75, nanos, zbt16*), somatic cells (*amh*) and spermatids (*odf2l, sept7a, cull3*). Plate coating did not enrich spermatogonia population, but strainers with a mesh of 5 µm could successfully retained spermatogonia. The sequential use of two 5 µm-strainer achieved a spermatogonia enrichment of 63.41 ± 4.51 % versus 25.97 ± 7.29 % for gelatine coatings. Gene expression analysis revealed that cell population retained on 5 µm strainers contained few somatic cells (low levels of *amh* expression) and was enriched in spermatogonia (high levels of *gfra, pou5f/oct4, notch1, mcm6*, *nanos, zbt16* and *ly75* expression and very low levels of *odf2l, sept7a* and *cull3* expression). These results suggest that strainer protocol could be a good option to enrich Senegalese sole spermatogonia. This method can be used in further studies of spermatogonial transplantation, cell culture or to better understand spermatogonia damage during cryopreservation procedures.

Keywords: differential plating, quality assay, germline stem cells, Solea senegalensis

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[P5]

EVALUATION OF SPERMATOGONIA DAMAGE AFTER CRYOPRESERVATION

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Cryopreservation of testicular germ cells (TGCs) offers a tool for the conservation and production of species with potential for aquaculture, due to the capacity of spermatogonia differentiating into gametes. Cryopreservation and transplantation of TGCs have been attempted in several fish species, but the evaluation of post-thaw quality has been disregarded since TGCs transplantation in most species is easily performed due to their high numbers. In the particular case of Senegalese sole, a species with semicystic spermatogenesis, it is very difficult to extract high numbers of spermatogonia from testis at any developmental stage, and therefore, it is important to develop an optimized protocol for the cryopreservation of these cells, guaranteeing high survival. In the present study, an evaluation of cryopreservation protocols for TGCs from Senegalese sole (*Solea senegalensis*) was performed. Several 12-months old fish juveniles were anesthetized with a lethal dose of phenoxyethanol (2,000 ppm, 5 min) for chirurgical testes removal. Each testis was divided in 4 fragments and several fragments were cryopreserved in PBS or L-15 based medium supplemented with 0.5% BSA and 5.5 mM glucose and 1.5 M DMSO or 1.5 M glycerol. Testes fragments were frozen in cryovials using a programmed biofreezer (Grant Asymptote, UK). Cell viability, lipid peroxidation and DNA fragmentation were determined in post-thaw isolated cells (40 ^{o}C, 2.30 min). Transplantation of germ cells was performed to check for cell capacity to incorporate in the gonadal *primordium* at early larval stages. For that, PKH26 stained cells were microinjected intraperitoneally into anaesthetized (0.01% MS-222) *S. senegalensis* larvae from 6 to 20 days post-hatching (dph). After transplantation, larvae were reared until 41dph according to the protocol established by our group. Protocol incorporating DMSO as cryoprotectant showed higher number of recovered spermatogonia, especially in samples cryopreserved with L-15+DMSO. Lipid peroxidation and DNA fragmentation were also significantly lower in this treatment. An important increase in oxidization was detected in samples containing glycerol as cryoprotectant, reflected also in terms of higher DNA damage. Transplantation of L-15+DMSO cryopreserved germ cells into larvae during early metamorphosis (10 dah, 5.2 mm) showed higher incorporation of cells than other larval stages. Testes cryopreservation proved to be a useful method to store Senegalese sole germline for further transplantation.

Keywords: oxidative stress, DNA damage, germ cells transplantation

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[P6]

STEPS TOWARDS ESTABLISHING A PRIMARY CULTURE OF ZEBRAFISH PREVITELLOGENIC OVARIAN FOLLICLES

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Novel methods for studying oogenesis are moving toward *in vitro* approaches that would allow detailed access to key molecular mechanisms governing it. In that regard, a reliable primary culture supporting differentiation and growth of fish oocytes would be valuable as an experimental model and a key step in future cryopreservation protocols. Due to complex metabolic and regulatory requirements of growing follicles, studies detailing their *in vitro* culture are rare, with most of them focused on very early- or late- stages of oogenesis. Here we report the first steps in establishing a culture of late previtellogenic ovarian follicles (translucent, 290-340 µm) isolated from zebrafish (*Danio rerio*). During tissue dissociation, follicles of size above 250 µm displayed distinct sensitivity to low concentrations (50 µg/ml) of trypsin and collagenase II. In comparison, both mechanical dissociation (gentle pipetting, fine-tip forceps) and use of hyaluronidase (1 mg/ml) resulted in high separation of follicles while preserving follicular layer integrity and oocyte survival. The best performing culture medium was 90% Leibovitz (L-15) supplemented with antibiotics, non-essential amino acids, insulin-transferrin-selenium, sodium pyruvate, ascorbic acid and 10 mM HEPES (pH 7.6); however, the majority of follicles did not survive past 48 h of culture. Addition of 4% common carp serum obtained from females treated with high doses of 17ß-estradiol notably improved the follicle survival, compared to serum-free media and other supplements, with follicles maintaining viability past 4 days of culture. In optimized media, the viability of oocytes was 80±6% after 24 h; however, this rate dropped to 42±9% with prolonged culture (> 96 h). The loss of viability was mainly attributed to the changes in integrity of the follicular layer, such as fragmentation of the *zona pellucida* and follicular cell hypertrophy, which implied initial follicular atresia. Measuring follicle diameter and lipid/lipoprotein staining showed no significant growth or accumulation of yolk vesicles. In addition, the expression of main genes involved in hormone biosynthesis and signaling (*cyp19a1a, fshr, esr1*), as well as lipid metabolism (*lpl, lpr13*), remained stable during short-term culture and comparable to freshly isolated previtellogenic ovarian follicles. Successful maintenance of follicles in culture and establishment of cytological and molecular markers to monitor their progress enables insight into their specific requirements and potential stimulation of vitellogenesis and growth *in vitro*.

Keywords: oocyte growth, in vitro growth, Danio rerio, vitellogenin, in vitro culture

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[P7]

REVISITING FIXATION OF TESTICULAR TISSUE AS AN IMPORTANT PREREQUISITE FOR MORPHOLOGICAL INVESTIGATIONS

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Neutral-buffered formalin (NBF) and Bouin's fluid (BF) are well known and most commonly used fixatives in histology. However, recent regulatory guidelines on reproductive toxicity tests have started to recommend a substitution of NBF for BF or similar fixatives [1]. As the picric acid in BF is a health hazard, requires intense rinsing for its removal, and can interfere with immunohistochemistry outcomes, other fixatives such as the modified Davidson's fluid (mDF) have quicky risen as alternatives. In fixation of mammalian testes, mDF displayed better morphological detail and immunohistochemistry outcomes than NBF and BF. The aim of this study was to test whether mDF can be used as a substitute for NBF and/BF in the fixation of fish testicular tissue.

Zebrafish (*Danio rerio*) testes were fixed in either 10% NBF, BF or mDF overnight (~16 h) at either 4 ℃ or room temperature (~23 ℃). Tissues were routinely processed, and sections were stained with H&E staining. BF and mDF displayed superior preservation of morphological detail compared to NBF, especially in nuclear detail which is of paramount importance for distinguishing various stages of germ cells. There was no difference between the fixation temperatures. Immunostaining for the commonly used germ cell marker vasa identified strong signal in germ cells of testes fixed in NBF and mDF, while staining was negative for the samples fixed with BF. Similar results were obtained in African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) testes. Obtained results indicate that mDF is a valuable substitute for the commonly used NBF and BF as it enables superior preservation of morphological detail, as well as good immunostaining outcomes.

Keywords: histology, immunohistochemistry, neutral-buffered formalin, modified Davidson's solution

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References:

[1] Latendresse JR, et al. (2002). Fixation of testes and eyes using a modified Davidson's fluid: Comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol Pathol.*

[P8]

TESTICULAR ORGANOIDS IN TELEOST FISH: CURRENT PROGRESS AND PERSPECTIVES

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During the last decades, several strategies have been developed to study spermatogenesis *in vitro*. However, most of the existing methods were not able to reproduce the cellular interactions as similar as in the native testis. Recently, the establishment of a culture system based on scaffolds would provide a 3D microenvironment to support testis cells growth and development, facilitating the *de novo* testis organogenesis and functioning. This artificial system, named as organoid, would permit a more detailed investigation of physiological testicular functions, including the interactions between germline stem cells (GSCs) and somatic cells, as well as mechanisms involved in male infertility (disease, ecotoxicology). Moreover, for species or individual with high genetic value, organoids would also allow the amplification of GSCs for the conservation of genetic resources and the regeneration of the cohorts of interest. Considering this background and the lack of testicular organoids in teleost fish, the current study aimed to develop a scaffold-based testis culture system in fish. The first approach consisted in developing an endogenous hydrogelscaffold from decellularized extracellular matrix (dECM) of rainbow trout immature testes. The second approach aimed to test different commercially available hydrogels (natural and synthetic) for establishing fish testicular organoids. In the first part, we successfully obtained dECM from immature trout testes without affecting its natural composition and structure, as shown by histochemistry, immunofluorescence, and scanning or transmission electron microscopy. These data open future possibilities to develop testicular organoids using a natural/endogenous scaffold. With regards to the second strategy, we showed that zebrafish testicular cells were able to form cell clusters when encapsulated into commercial hydrogels (Vitrogel or Matrigel). The use of testicular cells from different reporter lines demonstrated that these clusters were not derived from partial cell dissociation but were rather formed from spontaneous reaggregation of previously dissociated cells. The cell clusters encapsulated into Matrigel presented a more regular and round shaped morphology as compared to Vitrogel. We also observed that cell clusters changed in morphology, cell density and some exhibited bud formation during the culture period (19 days). Future studies will be required to further characterize the structure of these cell clusters and improve the culture medium to promote cell growth and *de novo* testis organogenesis.

Keywords: teleost fish, testicular organoids, stem cell, germinal niche, biotechnology

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[P9]

OOGENESIS IMPAIRMENT IN SWORDFISH (*XIPHIAS GLADIUS*) CAUGHT IN CENTRAL ADRIATIC SEA: DIFFERENCES BETWEEN MATURE AND IMMATURE FEMALES

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The swordfish (*Xiphias gladius*) is a cosmopolitan, highly migratory teleost species and an important fishing resource. Since the Mediterranean stock is considered in overfishing and declining (ICCAT, 2017), the evaluation of the gonadal and health status of immature and mature females during the reproductive and no reproductive season could be of great importance as well as the determination of the reproductive stage of catch's sizes. 48 swordfish females caught in the Central Adriatic Sea were collected between August and November 2021. For each animal, gonadal and total weight was measured and liver and gonad samples were properly stored for histological analysis. The histological determination of reproductive stage confirmed that 80-90% of landed females were immature, situation that could contribute to the collapse of the stock. In addition, the assessment of histopathological biomarkers of oogenesis impairment such as pre-vitellogenic atresia, necrosis, vacuolization of pre-vitellogenic oocytes and the presence of infiltrates were found in both immature and mature females but in different percentages on the basis of the reproductive period. At hepatic level, the histological analysis revealed the great occurence of single melanomacrophages (MMs) and melanomacrophage centres (MMCs), the two biomarkers of acute and chronic stress here considered. The results highlighted that the density and numbers of MMCs changed in relation to the months and sexual maturity of females. In fact, mature females caught in August showed a lower density of MMCs compared to mature females caught in September and November. Moreover, significant differences of density and number of MMCs between immature and mature females were found in September and November, while in August similar results were found. Furthermore, lipids density in the liver of mature females was unchanged during the three months of catches. A significant difference of lipids density was found only between immature and mature females caught in September. Concluding, the impairment assessed at gonadal level could be related to the sub-optimal health status of Mediterranean swordfish which probably engages energies to cope environmental changes and overfishing, rather than to reproduce.

Keywords: reproductive stage, histological analysis, melanomacrophages, liver, ovary

[P10]

FINE PHENOTYPING OF THE MICROPYLAR CELL IN MEDAKA

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In the eggs of most teleosts, a funnel-shaped canal in the chorion, located in the animal pole, called the micropyle, allows the fertilization of the eggs by guiding the spermatozoa to the oocyte. The micropyle originates from the differentiation of single granulosa cell in somatic follicular cells layer during oogenesis called the micropylar precursor cell (MPC). From stage 3 (according to Iwamatsu et al., [1]) of oogenesis the MPC differs from the other follicular cells. The MPC develops a cytoplasmic extension, which will give its characteristic mushroom shape to the MPC and will allow connection to the oocyte membrane. As demonstrated by Gay et al. [2], mutant *mir202* -/- females, exhibit a dramatically reduced fertilization rate, in comparison to wild-type (WT) control females. Our working hypothesis is that eggs originating from *mir-202* -/- females exhibit a non-functional micropyle due to dysregulated MPC differentiation during oogenesis. In this context, the aim of our study was to thoroughly phenotype MPC differentiation to better understand why eggs produced by medaka (*Oryzias latipes*) lacking miR-202 exhibit a reduced fertilization success.

Using scanning electron microscopy, we showed that 95% of micropyle of miR-202 -/- eggs were closed and thus non-functional, compared to 22% of the WT eggs. To further characterize the phenotype, we immunostained MPC makers, on ovarian sections, including acetylated tubulin, Taz, Phospho myosin, Phospho-S6 and Nup 107. We highlighted that among the eggs from miR-202 -/- females, different abnormal MPC phenotypes could be observed that were not present in wild-type controls including a discontinuous cytoplasmic elongation or an abnormal shape of the MPC. *In toto* immunostaining coupled with RNAscope (*ccnb1*, miR 202) allowed the orientation of the follicle to visualize the complete MPC in a single image and to verify the different phenotypes highlighted on sections. The next challenge will be to co-localize miR-202 and its possible targets in the MPC. Together, our data will provide a comprehensive vision of the phenotype of MPC differentiation in miR-202 mutant eggs.

Keywords: oogenesis, oocyte, micropylar precursor cell, miR-202, Oryzias latipes

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References:

[1] Iwamatsu T, et al. (1988). Oogenesis in the Medaka *Oryzias latipes*: Stages of Oocyte Development : Developmental Biology, *Zoolog Sci*.

[2] Gay S, et al. (2018). MiR-202 controls female fecundity by regulating medaka oogenesis. *PLoS Genet.*

[P11]

PROTEOMIC PORTRAIT OF RAINBOW TROUT OVARY IN RESPONSE TO TRIPLOIDIZATION

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Rainbow trout are characterized by high amenability to chromosome manipulations during early development to produce androgens, gynogens, or sterile triploids, which are relevant in aquaculture. An understanding of the mechanisms affecting oogenesis and those resulting in diminished gonadal development in triploids is an important area of scientific study. Therefore, the aim of this study was to compare the ovary proteome from triploid (3N) and diploid (2N) rainbow trout females using tandem mass tag (TMT) peptide labelling coupled with liquid chromatography–mass spectrometry (LC–MS/MS) to identify differentially expressed proteins (DEPs) between 2N and 3N. The potential biological role of DEPs was also explored using comparative functional gene set enrichment to designate the potential protein markers of 3N sterility. We identified a total of 3899 DEPs ($q < 0.05$), including 956 downregulated and 2943 upregulated proteins in 3N ovaries compared to 2N ovaries. The identified clusters for downregulated proteins in 3N included lipid metabolic process, hormone biosynthesis, transport and exocytosis, cell junction organization and sperm-egg recognition, which can result in (i) the disruption of lipid, steroid hormone and vitellogenesis processes, (ii) impaired amino acid uptake, (iii) defects in cell–cell junctions reflecting tissue abnormalities and aberrant gonadal morphology, and (iv) impaired functions related to maintaining the proper structure of the vitelline envelope, oocyte formation and fertilization competence. With respect to upregulated proteins in 3N ovaries, RNA processing, cell cycle, cytoskeleton organization and regulation of metabolic process were the primary clusters, which may reflect (i) the disturbance to RNA processing responsible for impairment of ovary development in triploid rainbow trout, (ii) disruption of spindle formation, incorrect chromosome alignment and segregation during meiotic division, (iii) increase in regulatory mechanism of apoptosis, which in consequence can provoke abnormal gonadal development and sterility, and (iv) enhanced ubiquitin–proteasome system governing aberrant gonadal morphology in 3N females and oocyte maturation. The obtained results provide an accurate portrait of molecular changes in rainbow trout ovaries in response to triploidization and may contribute to a better understanding of the mechanisms responsible for impaired oogenesis in rainbow trout triploid females, which in turn leads to their sterility.

Keywords: triploidy, fish, gonads, proteome, mass spectrometry

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[P12]

STRUCTURAL AND MACROMOLECULAR CHARACTERIZATION OF *MUSTELUS MUSTELUS* GAMETOGENESIS: NEW INSIGHTS BY COUPLING HISTOLOGY WITH FOURIER TRANSFORM INFRARED MICROSPECTROSCOPY

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In oceans and seas around the world, Chondrichthyes, are facing an intense and rapid decline. Faced with this situation, very little has been done for the conservation of these species, especially in the Mediterranean, where only a limited number of species benefit from protective measures. This lack of management and protection measures has been attributed over the years to the scarce scientific knowledge available for the different species. The aim of this study is to improve the knowledge and identify macromolecular changes underlying gametogenesis (both oogenesis and spermatogenesis) in the common dogfish, *Mustelus mustelus*, an important shark with a commercial value fished in the Mediterranean Sea but considered Vulnerable (VU) by IUCN.

In this work, histology coupled with FTIR FPA techniques were used to deepen knowledge of gametogenesis of *Mustelus mustelus*. Regarding spermatogenesis, 7 developmental stages of germinal cells, from spermatogonia to mature spermatozoa, were described and characterized. In addition, six developmental stages of spermatocysts were described together with how spermatocysts change and mature within the testicle, and how germ cells and Sertoli cells relate and mature together. Furthermore, for the first time, sharks male gonads were subjected to spectral analysis by FT-IR for a characterization of the macromolecular composition and maturation of the germinal and somatic components of the spermatocysts. A focus on germinal cells evidenced how the maturation process consists of changes in particular related to lipids (in terms of amount, length of the chains and saturation levels) and related to DNA (in terms of amount and methylation rate). Regarding oogenesis a focus on the spectral characterization of *Mustelus mustelus* oocytes at different stages of development (from oogonia to mature oocytes) was done highlighting for the first time the macromolecular changes of Zona Radiata, ooplasm and yolk vesicles during follicle growth. In conclusion, these results represent a starting point for future quality assessment of shark gametes.

Keywords: spermatogenesis, oogenesis, vitellogenesis, shark

[P13]

DIETARY PHYTOESTROGENS EFFECT ON SEX-RATIO AND KEY SEX-RELATED GENES EXPRESSION IN STURGEON

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Phytoestrogens are compounds of estrogenic properties, present in high concentrations in sturgeon diet due to common utilization of the soybean and its derivatives for aquafeeds production. The mechanism of those compounds and their effect on sex-related gene expression and gonad differentiation is, so far, poorly understood. Therefore, this study aimed to examine the expression of the *dmrt1*, *sox9*, *amh*, *foxl2*, *cyp19* and *vtg* genes in the gonads of one-year old Russian sturgeons exposed for 265 days to daidzein, genistein and coumestrol administrated as feed additives in the concentration of 10,000, 500 and 10 mg kg^{-1} feed, respectively.

Based on the histological analyzes of the gonads in the Control group sex distribution was almost equal to 1:1 (females to males), however, one intersex with dominating male component was also noted. In the group fed with addition of daidzein the sex distribution was similar to that observed in the Control group, although with an increase number of intersex individuals. In the groups fed with feed with addition of genistein (Genistein group) and coumestrol (Coumestrol group), no males were observed. The Genistein group was composed of mainly females with sex ratio 9:1 (females to intersex individuals) and similarly in the Coumestrol group mainly females were detected, yet, with increased share of intersex individuals, presenting sex ratio 4:1 (females to intersex individuals). The most notable difference in sex-related genes expression observed in the gonads was a significant down-regulation, in the individuals fed with phytoestrogen-supplemented feed, which suggests that the studied phytoestrogens may inhibit molecular pathways responsible for sturgeon sex differentiation. The abovementioned findings suggested that phytoestrogens can cause significant changes in the expression profiles of sex-related genes resulting in molecular pathway alterations, which can ultimately lead to sex differentiation disturbances and therefore to altered sex-ratio in sturgeon stocks.

Keywords: coumestrol, daidzein, genistein, histology, real-time PCR

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[P14]

PATHOLOGIES OF STURGEON GAMETOGENESIS

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There is a substantial lack of knowledge regarding gonad pathologies in sturgeons, even though the occurrence of such disorders poses a significant threat to the future of both commercial and conservation farming. Eleven years of our research on gonad differentiation and development of Russian and Siberian sturgeon, significantly contributed to sex identification in early developmental stages, but also allowed to identify impairments in female and male germinal tissue development. A detailed histological analysis of gonadogenesis conducted across over 4 years of the specimens' lifetime, revealed multiple gonadal disorders, many of which can contribute to decreased fish fertility or even sterility. Some of the identified pathologies are believed to be related to dietary phytoestrogens, hypoxia, stress or infections, however, the main causes are not yet recognized since there is still insufficient knowledge on factors which determine differentiation and development of gonads in sturgeons.

The most commonly described gonadal pathology is related to biased sex ratio in the sturgeon stocks, which is the result of masculinization or feminization. This, also often causes appearance of intersex individuals. Intersexes with well-developed female and male gonadal components may undergo self-fertilization which drastically increase inbred in fish stocks. The second most commonly noted histopathology was chronic gonaditis with large lymphocytic infiltration. In the extreme cases over 90% of the gonad was occupied with inflammatory cells which can decline fish fertility. The third most frequently observed histopathology, namely atresia of oocytes and ovarian follicles, has also the most severe consequences. In such cases atretic germinal tissue was replaced by fat tissue and in consequence almost whole gonadal volume was filled up with it. This pathology can drastically decrease roe production and finally cause almost complete female infertility. Less common pathologies that were found in sturgeon gonads concerned inhibition of gonadal tissue development. For example, some specimens at the age of over 2 years still had undifferentiated gonocytes as the only type of germinal cells in the gonads. In a few cases also arrested spermatogenesis was noted. This type of pathology can cause extended maturation, depending on when such developmental block will be released. The rarest observed pathology concerned double oocyte development in one follicle. In this case there might be an issue with egg fertilization or twin embryo development, however such observations were not published up to this date.

Keywords: intersex, gonaditis, follicular atresia, arrested gonocyte differentiation, double oocyte development

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[P15]

EFFECT OF TWO COPPER NANOPRODUCTS AND THE IONIC FORM ON RAINBOW TROUT (*ONCORHYNCHUS MYKISS* W.) SPERMATOZOA MOTILITY

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The amount of the nanoproducts released into the environment is a growing threat to organisms. There is little data on the effects of nanoproducts made of different compounds of the same element on biological objects. The copper nanoproducts are widely utilized due to their antimicrobial properties in feed industry, cosmetics, medical drug designing, and numerous others applications [1]. Spermatozoa as a cell is a good model for studying the ecotoxic effects of nanoproducts and other chemicals on cells [2]. The direct effect of two copper nanoproducts of Cu NPs and CuO NPs and copper ions ($CuSO_4$) on spermatozoa motility was previous investigated by our group by adding pollutants to the environment activating the gametes. In the study high concentrations of pollutants were used (up to 500 mg Cu L^{-1}). More harmful effect of ionic form than nanoparticles was obtained in the study while diverse effects of the nanoproducts was not clear [3]. The present study aimed to analyse the effect of these two copper nanoparticles and metal ions on rainbow trout spermatozoa motility parameters after long-term exposure.

Nanoproducts of Cu and CuO (Cu NPs, CuO NPs) of primary particle size around 50 nm and ionic solution of $CuSO_4$ were used for the study. Suspension of concentration 0, 1, 5, 10, 25 and 50 mg Cu L^{-1} as Cu NPs, CuO NPs and $CuSO_4$ was dissolved in an artificial seminal plasma. Milt was mixed with prepared solution and stored in a fridge for 96 h. At the defined incubation time, spermatozoa were activated for movement. Six motility parameters were evaluated using an automated system (CASA).

Copper nanoproducts made from different compounds have a different effect on cells. Cu NPs was more harmful than CuO NPs. The effect of Cu NPs was similar to an ionic form of $CuSO_4$. When incubated, the copper nanoproducts and ionic form exert a slightly positive effect on spermatozoa velocity, linearity, and motility duration, particularly in the initial hours of storage.

Keywords: Cu nanoproduct, CuO nanoproduct, metal ions, ecotoxicology, sperm motility parameters

References:

[1] Cioffi N, et al. (2005). Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. *Chem Mater.*

[2] Shaliutina O, et al. (2021). Using fish spermatozoa in *in vitro* toxicity tests: A potential toxicology tool. *Aquaculture*.

[3] Kowalska-Góralska M, et al. (2019). Effect of copper nanoparticles and ions on spermatozoa motility of sea trout (*Salmo trutta* m. *trutta* L.). *Aquat Toxicol.*

[P16]

MOTILITY AND VOLUME REGULATION OF PIKEPERCH (*SANDER LUCIOPERCA*) SPERMATOZOA UNDER DIFFERENT OSMOTIC AND IONIC CONDITIONS

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In pikeperches, as in many other species, sperm quality is a prerequisite for fertilization success and efficient natural and artificial reproduction. The capacity of fish spermatozoa to be activated and tolerate environmental changes, such as osmolality, ion composition, external pH, and temperature, during the motility period is related to the sperm quality and greatly contributes to fertilization success. The current study aimed to investigate the role of environmental osmolality and ion composition in sperm motility regulation and their correlation to different sperm quality parameters. Therefore, semen characteristics such as semen volume, osmolality and ion composition of seminal fluid (SF), spermatozoa concentration and fatty acids (FA) composition, as well as sperm motility (percentage of activated cells, their curvilinear velocity (VCL), and linearity(LIN)) were analyzed and compared between males. We observed high variation between males in quality characteristics such as milt volume, SF osmolality, SF ions concentration, and spermatozoa concentration. That partly could be explained by the high risk of sperm urine contamination during sampling in this species. The percentage of sperm motility under various osmolarity and ion composition was also different between males. However, no difference in VCL or LIN was detected. Sperm samples with the highest content of Palmitic (C16:0) and Palmitoleic (C16:1) acids show the lowest percentage of motility, suggesting these FA could be involved in the spermatozoa motility activation process and used as quality markers. We found that pikeperch sperm motility is always accompanied by swelling. Even in hypotonic media, cells could keep their volume if spermatozoa motility is arrested by high K^+ concentration. Our results suggest that pikeperch spermatozoa activation is not fully controlled by osmotic shock. Instead, motility activates when extracellular ion concentration decreases during spawning when spermatozoa are mixing with environment in fresh water.

Keywords: spermatozoa swelling, semen quality, osmoresistance, lipid composition analyses

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[P17]

FROM BRAIN TO SPERM: HOW PSYCHOACTIVE POLLUTANTS CAN AFFECT FISH SPERM FUNCTION

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There is a growing concern about the occurrence of pharmaceutical active compounds (PACs) in aquatic environment. Especially psychoactive pharmaceuticals are of concern, since they are widely prescribed with continuously increasing trend, resulting in ubiquitous occurrence in surface waters. Recent studies indicate adverse effects of psychoactive drugs on aquatic life at environmentally relevant concentrations. Pathological and behavioural changes in fish and cray fish caused by exposure to psychoactive compounds are reported. Despite this, very little is known about the mechanisms of the above-described changes in aquatic organisms, including fish. According to the theoretical model of the effects of human pharmaceuticals in fish, they will exhibit similar biological effects across species (e.g., human and fish), if the molecular target has been conserved and effective drug concentrations reach the blood plasma. Psychoactive drugs have a specific action on one or more neurotransmitters (NT) or neuroreceptors which are present in neuron cells both in humans and fish. Individual targets are well conserved suggesting that psychoactive drugs in fish act through similar mechanisms as in humans. Information about contaminant-induced changes in brain NTs can be crucial, because it may link behaviour and physiology in the exposed fish.

Neuronal signalling is important not only for brain function, but also the sperm function is regulated by signals, several of which correspond to neurotransmitters that activate the transduction signalling implicated in molecular control of sperm physiology. Invertebrate and mammalian spermatozoa express receptors for many neurotransmitters and neuromodulators, e.g., acetylcholine, γ-aminobutyric acid, serotonin, norepinephrine, and dopamin. Sperm cells also express receptors for psychoactive drugs, e.g., nicotine, cocaine, opioids and cannabinoids. Information on the presence of these kind of receptors in fish sperm is limited, nevertheless opioid receptors were confirmed in sea bream and sea bass, some recent reports also suggest participation of endogenous opioid peptides in regulating reproductive axis of medaka and carp. Some of important NTs may be involved in sperm function and constant exposure of fish to psychoactive compounds which are ubiquitous in the aquatic environment may lead not only to behavioural changes, but also affect sperm functioning and consequently reproduction. Thus, in our future experiments we aim to track the changes of NTs levels in brain of fish exposed to psychoactive drugs and to investigate if those changes can be involved in sperm functioning. We plan to assess the presence of receptors to certain NTs in fish sperm and gonads and examine the effects of psychoactive drugs on the motility and quality of fish spermatozoa in vitro, in vivo and in situ.

Keywords: neurotransmitters, psychoactive drugs, emerging pollutants, sperm function

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[P18]

NOVEL MASS SPECTROMETRY-BASED METHODS FOR THE ASSESSMENT OF SPERM QUALITY

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Describing spermatozoa metabolism by evaluating metabolites contents is one of the methodological approaches in spermatology. This approach provides valuable information about the processes occurring in spermatozoa under different physiological and experimental conditions, such as sperm motility, reactivation, capacitation, maturation, and other *in vitro* manipulation. Most fish species reproduce externally, and spermatozoa activate their motility only after being released to the open water during spawning. In such a harsh environment, spermatozoa receive osmotic damage from fresh/seawater, commonly limiting their lifetime to several minutes. During such a short period of motility, the slow metabolic activity can't maintain the energy level of the spermatozoa, suggesting that the activity of the spermatozoon relies only on energy sources accumulated before. Thus the determination of macroergic metabolites in fish spermatozoa is of high importance and could directly correspond to the physiological status of spermatozoa and characterize their quality. The development of a rapid method for the quantitative assessment of a large number of metabolites also provides a good opportunity to understand species-specific metabolic energy pathways. Another important group of compounds is neurotransmitters (NT). Sperm function is regulated by signals, several of which may correspond to NTs that activate the transduction signaling implicated in the molecular control of sperm physiology. Most frequently used approaches allow quantifying only one single substrate, so limited data can be obtained. Therefore, simultaneous analysis of several interrelated metabolites using liquid chromatography coupled to mass spectrometry (LC/MS) is of great interest as it allows the acquisition of more information from a single measurement, which consequently can be used for the assessment of the role of certain compounds in sperm metabolism. Novel LC/MS analytical methods allow sensitive and selective identification and quantification of biomolecules in complex biological matrices. Moreover, using high-resolution mass spectrometry allows to do non-target analysis and identify metabolites involved in sperm function.

We present analytical methods for the determination of macroergic phosphates and neurotransmitters in fish sperm. Reversed-phase and HILIC chromatography were used to separate target compounds, followed by the detection with a hybrid Q Exactive™ HF Orbitrap mass spectrometer. The methods have good linearity, repeatability, the limit of quantification, and trueness parameters and allow the robust analysis of a wide range of metabolites in fish sperm.

Keywords: LC/MS, macroergic phosphates, neurotransmitters, sperm

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[P19]

EVALUATION OF OSMOTIC PUMPS AS A METHOD TO INDUCE SEXUAL MATURATION IN EUROPEAN EEL (*ANGUILLA ANGUILLA)* MALES AND FEMALES

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The European eel is a commercially valued species for aquaculture. Traditionally, the sexual maturation of the European eel has been induced by weekly hormonal injections, that requires repetitive handling which causes stress to the fish. In this study, the objective was to validate the osmotic pumps (OP) as an alternative method to induce the sexual maturation and the production of gametes in both males and females.

Regarding males, eels were divided in Control, OP-100 and OP-200 groups (n=10/group). The control group received weekly injections of hCG_{rec} (1.5 IU/g fish) for 15 weeks. The OP-100 and OP-200 groups were implanted with hCG_{rec}-loaded OP (ALZET 1004 and 2006, respectively). Sperm samples were collected weekly by stripping and the gamete quantity and quality were assessed using software CASA. Concerning females, eels were divided in Control (n=11) and OP-2ML4 (n=10) groups. The control group received weekly injections of carp pituitary extract (CPE; 20 mg/kg fish) for 15-20 weeks. The OP-2ML4 group was implanted with CPE-loaded OP ALZET 2ML4. Oocyte samples were collected weekly using a cannula. The ovulation of matured females was induced using a DHP injection and the eggs were collected by stripping to do fertilization trials. Biometric parameters were evaluated for both males and females.

In males, the OP induced the sexual maturation and spermiation, but sperm had not enough amount of spermatozoa (volume, density) with acceptable quality (motility and velocity) to replace the traditional method. In females, the OP induced the sexual maturation in 80% of females, and the 50% showed an unusual early maturation between 4^{th} and 10^{th} week. Some females were successfully induced to spawning, reaching embryos stage, but no hatching was obtained. Therefore, this study showed that the use of OP can be useful in the attempt of inducing sexual maturation, spermiation and ovulation on European eel. In the case of males, results showed that OP have not still enough potential to produce acceptable values of sperm. However, in females, the OP were able to generate spawning females so this method could become a viable alternative for eel hatchery procedures.

Keywords: spermiation, ovulation, human chorionic gonadotropin hormone, carp pituitary extract, hormone release system

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[P20]

EVALUATION OF THE REPRODUCTIVE TRAITS OF CAPTIVE BREEDING POPULATIONS OF ENDANGERED LEUCISCID SPECIES FROM THE IBERIAN PENINSULA

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Populations of freshwater fish species endemic to the Iberian Peninsula have been declining since the mid-20th century, and the captive breeding of highly endangered species is considered to be a useful tool to restock declining populations. A pioneer project of supportive breeding of critically endangered fish started in 2007 at the Aquário Vasco da Gama (AVG), and this work aims to show the reproductive status of the breeders which make up the current captive broodstoks.

Populations of different leuciscid species (*Anaecypris hispanica, Iberochondrostoma lusitanicum* and *Achondrostoma occidentale*) were sampled at AVG during the spring of 2022. Breeders were counted and sexed, and males were stripped to check for the presence of spermatozoa. The sperm volume was assessed visually, and spermatozoa motility was assessed by a CASA system. Sperm samples were classified into four classes based on the percentage of motile cells: C-I ≤ 25%, C-II = 25–50%, C-III = 50–75%; and C-IV > 75%.
The captive population of *A. hispanica* consisted of 63 individuals and showed a 40% of spermiating males, with an average volume of 5-10 µL. The histogram of sperm quality reported that 15% males had sperm motility of C-II, 50% of males had sperm motility of C-III and, finally, 35% of males had sperm with the high-quality class (C-IV). The population of *I. lusitanicum* consisted of 599 individuals and showed 93% of spermiating males, with an average volume of 15-20 µL. The histogram of sperm quality reported that most part of the males had good sperm quality belonging to C-III and C-IV class (26% and 71%, respectively), while just 1 male showed bad quality sperm (C-II). The captive population of *A. occidentale* consisted of 193 individuals, showing a 62% of spermiating males with an average volume of 20-25 µL. The histogram showed that 6% males had sperm motility of C-I, 26% of males had sperm motility of C-II, the most part of the males (45%) showed a sperm quality of C-III and, finally, 23% of males had sperm with the high-quality class (C-IV). Since the project began in 2007, more than 12,000 fish of these three critically endangered species have been released to restock the populations from which the respective wild breeders were caught. All captive fish were released after a maximum of three consecutive generations in captivity, and new stocks were established with wild adults from the target populations, to avoid the negative effects of inbreeding and genetic drift on the original genetic pool.

Keywords: gamete quality, ex-situ conservation, sperm kinetics

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[P21]

COMPARATIVE ANALYSIS OF SEMEN QUALITY BETWEEN SEX REVERSED FEMALES (NXX), ANDROGEN TREATED MALES (NXY) AND NORMAL MALES (XY) OF EURASIAN PERCH (*PERCA FLUVIATILIS* L.)

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Sex reversal has been used as a reproductive strategy to produce genetically and phenotypically monosex fish populations, which is of interest in commercial aquaculture due to i.e., higher growth rates or reduce sexual growth dimorphism. Sperm from sex reversed homogametic females (XX) could be used for fertilize ova from normal females (XX), which results in the production of 100% female progenies. Analysis and verification of semen quality prior to its use in artificial propagation is critical to obtain high rates of fertilization, especially after sex reversal procedure. Therefore, the aim of our study was to compare the quality of semen obtained from sex reversed females (nXX), androgen treated males (nXY) and normal males (XY) of Eurasian perch.

Sex reversed females (nXX) and males (nXY) of Eurasian perch were produced via dietary treatment by 20 mg OHA kg^{-1} (11β-hydroxyandrostenedione) during the sex differentiation period. Normal males (XY) originated from pond-reared perch stock. 7 days before sperm collection, 5 males from each group were stimulated for spermiation with 300 IU kg^{-1} of hCG. Semen from XY and nXY males was collected by striping while from neomales (nXX) testicular semen was used. The osmolality of seminal plasma was determined using a vapor pressure osmometer and sperm concentration was estimated spectrophotometrically. Sperm kinetics were examined with CASA system (SCA). CASA parameters analyzed were: MOT–percent of motile sperm (%), VCL–curvilinear velocity (µm s^{-1}), VAP–average path velocity (µm s^{-1}), VSL–straight line velocity (µm s^{-1}) and ALH-amplitude of lateral head displacement (µm). All analyses were performed at a significance level of 0.05 using one-way ANOVA followed by HSD Tukey post hoc test.

No significant differences in percent of motile sperm (MOT), sperm concentration and osmolality of seminal plasma between sex reversed females (nXX), androgen treated males (nXY) or normal males (XY) of Eurasian perch were found. All velocity CASA parameters (VCL, VSL and VAP) was significantly higher in semen obtained from XY than in semen from nXY and nXX perch. A similar result was observed in amplitude of lateral head displacement parameter. In conclusion, we consider that OHA is effective to produce functional sex reversed females (XX) whose sperm can be used for the production of all-female population of Eurasian perch. However, semen from hormonally treated fish is characterized by lower CASA parameters than semen from normal males, which may affect final effectiveness of the planned reproductive procedures.

Keywords: 11ß-hydroxyandrostenedione, neomales, CASA analysis

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[P22]

AGING OF PIKEPERCH EGGS INDUCES CHANGES IN THE EXPRESSION OF PROTEINS INVOLVED IN GENOME STABILITY AND EGG QUALITY

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Pikeperch (*Sander lucioperca*) is one of the species with the highest potential for European aquaculture diversification and year-round production. The egg ageing, i.e. loss of their viability after ovulation, is one of the limiting factors in controlled reproduction in this species, and biological processes involved are so far poorly understood. The aim of this study was the identification of proteomic markers underlying the egg ageing process in pikeperch with use of tandem mass tag (TMT) peptide labeling coupled with liquid chromatography–mass spectrometry (LC-MS/MS) quantitative proteomics technique. Eggs were sampled at 1 h (control, n=6) and 5 h (aged group, n=6) following ovulation. Time of 5 h was chosen, since eggs maintained their cellular integrity with no morphological features of atresia, but their fertilizing ability was significantly lower (47.8%) than control group (89.7%). We identified four differentially expressed proteins (q–value < 0.05) between control and aged groups: upregulated proteins DNA replication complex GINS protein SLD5 (gins4) and transcriptional regulator ATRX isoform X2 (atrx), as well as downregulated proteins 39S ribosomal protein L10 (mrpl10) and dnaJ homolog subfamily B member 14 (dnajb14). The ATRX and GINS4 were found to have a role in the maintenance of genomic stability, normal cell cycle progression and in replication stress prevention. In turn, *DNAJB* and MRPL10 are associated with egg quality and premature ovarian failure in higher vertebrates. It can be suggested that enhanced expression of artx and gins4 in pikeperch aged eggs is one of the components of protective response to tackle DNA lesions in order to maintain their genome integrity. On the other hand, changes in expression of dnajb and mrpl10 proteins could be linked with lower quality of eggs resulting in reduced fertilizing ability. However, the proposed role of those proteins in aging of pikeperch eggs is speculative and needs further studies, aimed to confirm the participation of identified proteins in preventing genome instability and in egg quality. Moreover, since ovulated egg is considered quiescent translationally, the increase in protein abundance after 5 h require verification. The identified proteins can be potential predictive markers of the initial stage of aging, where eggs are without apparent abnormalities, but with already lowered fertilizing capacity.

Keywords: mass spectrometry, TMT, proteomic, fish, egg quality

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[P23]

VITAMINS DIET SUPPLEMENTATION ENHANCES SPERM QUALITY IN GILTHEAD SEA BREAM

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Oxidative stress (OS) represents one of the main damages suffered by spermatozoa, which can be caused by several external factors. Yet, nutrition plays an important role in the control of oxidative events, by supplying adequate compounds that are transferred into the germline. Fish sperm is particularly prone to suffer OS, due to the high content in polyunsaturated fatty acids present in their membranes, and this can lead to a reduction in several spermatozoa functions, impairing sperm motility and cell viability, and later DNA integrity. This OS can be counteracted by the antioxidant system present in sperm. Therefore, antioxidants provided in the diet have a crucial role and are especially important during gametogenesis and spermiation. Recently, diet-derived antioxidants proved to modulate seminal plasma micro-RNAs profile present in the extracellular vesicles (EV), influencing sperm quality and progeny. The present study aimed to define a diet to improve gilthead seabream sperm quality to further explore a putative modulation of diet in EV profile. For this purpose, 3 groups of 21 fish were fed with 3 different diets: a) control diet, b) diet supplemented with vitamins C and E (VitC+E), and c) diet supplemented with selenium and zinc (Se+Zn). After 4 months of daily feeding, sperm was collected from each group to assess sperm quality parameters: i) spermatozoa motility by CASA; ii) lipid peroxidation by MDA quantification; iii) reactive oxygen species (ROS), iv) viability and v) cell apoptosis by flow cytometry and vi) DNA integrity by Comet assay. The group fed with VitC+E diet presented significantly higher progressive motile spermatozoa (PM) at 30 s post-activation (22.23 ± 6.17 %) in comparison with the control (19.43 ± 8.79 %) and Se+Zn (16.10 ± 7.62 %) groups. Significantly lower lipid peroxidation was observed in the VitC+E group (29.17 ± 12.26 uM) in comparison with the control group. No significant differences were observed in cell viability, ROS, apoptosis, and DNA integrity among groups. These results suggest that vitamins supplementation enhanced sperm quality in terms of motility and reducing the risk of lipid peroxidation protecting the bio-membranes from degenerative damage. Further relevance on the association of EV miRNAs profile with sperm traits and offspring quality will be investigated.

Keywords: gamete quality, antioxidant supplementation, oxidative stress, Sparus aurata

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[P24]

EFFECT OF ACCESSORY GLANDS ON SPERM PERFORMANCE OF LUSITANIAN TOADFISH, (*HALOBATRACHUS DIDACTYLUS*)

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Reproductive accessory organs are glands or ducts that do not produce gametes but are closely associated with testes. In fish, various functions have been attributed to these organs. In the Lusitanian toadfish (*Halobatrachus didactylus*), nest guarder males accessory glands undergo seasonal changes in weight and fluid production in parallel to the seasonal profile of gonadosomatic index. The role of these accessory glands in the reproductive tactic is not yet fully understood, but so far it is known that gland fluids, which runs abundantly from urogenital orifice during reproductive season (May-July), contain mucosubstances and proteins, a diversity of steroids, and highly potent odorants, suggesting that they should have an important role in the breeding process in different ways. In the present study, we investigated if these glands may also have a role in sperm enhancement in the Lusitanian toadfish. Reproductive mature fish (captive and wild) were anaesthetized and sacrificed to collect the testis and accessory glands (anterior gland - AG and posterior gland - PG). Spermatozoa was extracted from the testis and the glandular liquid obtained by slicing the organs and centrifuged to collect the supernatant. Sperm kinetic parameters, straight-line velocity (VSL), curvilinear velocity (VCL), as well as total motility (TM), progressive motility (PM) and linearity (LIN) were analysed using CASA system. Pure sperm and sperm diluted in three concentrations of each glandular liquid were activated with seawater. Kinetic parameters were recorded at 15, 45, 30, 45, 60, 120 and 180 seconds after activation. No motility was found in sperm diluted in the anterior or posterior gland secretion or both combinations, however spermatozoa was activated in the presence of both glands and seawater. There were differences between both captive and wild stocks in terms of sperm motility where wild stock maintained spermatozoa motility for longer period. Both glands significantly increase sperm velocity (VCL and VSL) in wild stock from 60 s after activation onwards, comparing to sperm without glands. These results suggest that substances secreted by accessory glands, mainly by AG, can prolong motility in guarder males ejaculate which may be an advantageous strategy to cope with long duration of female spawning while simultaneously perform nest guarding duties. The differences found between captive fish and wild fish suggest a potential effect of captivity conditions (e.g. environmental factors, food) on gonadal development. This work suggests that accessory glands of the Lusitanian toadfish male guarders, in addition to other functions (steroidogenic, pheromonal), enhance sperm performance during spermiation or during fertilization process. Further studies would be useful to completely understand the multi-faceted role of these organs in the reproductive process.

Keywords: sperm quality assessment, testicular sperm, cell motility, motility enhancement

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[P25]

OPTIMIZATION OF REPRODUCTIVE TECHNIQUES FOR SEA URCHIN (*PARACENTROTUS LIVIDUS*) *IN VITRO* REPRODUCTION

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Sea urchin gonads are considered a food delicacy in many countries around the world. This increase in interest in sea urchin roe is encouraging the aquaculture industry to develop a new lucrative product and to protect and restore natural populations. New research must ensure optimization of the manufacturing costs by improving reproductive management. In this sense, the selection of quality breeders, spawning induction and fertilization, larval cultivation, post-larvae settlement, juvenile cultivation, fattening and refinement of the gonads for consumption must be achieved. Furthermore, the optimization of *in vitro* reproductive techniques is also a crucial step for an efficient production. Cryopreservation and preservation are technologies that constitute alternative techniques that contribute to a more efficient management of resources of the purple sea-urchin (*Paracentrotus lividus*). The main goals of this study were to evaluate the quality of gametes from wild specimens caught in two different locations (South and North of Portugal) and to compare wild and two different aquaculture produced generations (F1 and F2). Moreover, optimization of *in vitro* fertilization protocols and long- and short-term sperm preservation procedures were performed. Sperm quality was evaluated through cell viability, motility parameters and DNA fragmentation. Significant differences in breeder selection were found, indicating that domestication have a positive influence on *P. lividus* sperm quality. *In vitro* assays with different sperm to egg ratios showed that 20000 spermatozoa/oocyte had the highest percentage of fertilization. When compared to cryopreservation, the short-term preservation method (4 ^{o}C) showed higher cell viability during the first six days, and none of the methods showed signs of DNA damage. As main conclusions, it is possible to infer that by using an optimized ratio of spermatozoa:egg, high fertilization rates can be obtained from animals born in captivity. In addition, sperm preservation at 4 ^{o}C seems to be a suitable method for sea urchin short-term reproductive management.

Keywords: echinoderms, sperm quality, in vitro reproduction, cryopreservation, breeders selection

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[P26]

COMPARATIVE STUDY ON SPERM QUALITY IN THREE FLATFISH SPECIES: HALIBUT, SENEGALESE SOLE AND TURBOT

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The control of gamete quality is a major issue for the aquaculture industry. However, some broodstocks born and reared in captivity still present some problems linked to factors such as disorder in gametogenesis, nutritional deficiencies, or lack of reproductive behaviors. Several problems detected in sperm quality have been identified as being caused by a deficiency in certain components in the diet. In flatfish species, where most fertilization events occur artificially, sperm quality is a very important aspect in broodstock management. Therefore, the main objective of this study was to characterize sperm quality in several broodstocks from three different flatfish species, Senegalese sole, Atlantic halibut and turbot, held at farm facilities, in order to develop nutritional strategies to ameliorate sperm quality. Turbot farming is still hampered by variable larval survival rates, due in part to the quality of breeders and gametes, which could be significantly improved by antioxidant and immune stimulating raw ingredients supplemented in the diets. Halibut males produce high amounts of sperm but with a variable quality between males and with a decreasing quality towards the end of reproduction. Senegalese sole have a similar problem with high variation in male contribution. In order to characterize sperm quality under production conditions, sperm from halibut was collected in Sognaqua farm; sperm from turbot was collected in FLATLANTIC and sole sperm was collected at El Toruño. Sperm quality parameters were evaluated, namely i) spermatozoa motility by CASA; ii) lipid peroxidation by MDA quantification; iii) reactive oxygen species (ROS) quantification, iv) viability and v) DNA integrity by Comet assay. Motility results showed a total of 35.8 % motile spermatozoa in halibut vs 66.0 % in turbot and 64.8% in Senegalese sole. VCL was 51.8 µm/s in halibut, 112 µm/s in turbot and 120.25 µm/s in Senegalese sole. As to viability, high values were registered in all 3 species, especially in turbot and halibut with sole males showing only 72% viable cells. Lipid peroxidation analysis revealed MDA levels of 3.4 µM/Million spermatozoa in halibut, while in turbot and sole these values were higher (24.3 and 278.5 µM/Million spermatozoa, respectively). ROS determination revealed that sole had higher levels compared with other species, with turbot being very resistant to ROS production. Improvement of sperm traits could be attained by diet supplementation, which should be design taking into consideration these results.

Keywords: cell motility, lipid peroxidation, cell viability, DNA fragmentation

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[P27]

OPTIMIZATION OF THE MEASUREMENT OF IDE (*LEUCISCUS IDUS*) SPERM MOTILITY USING CASA SYSTEM

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Sperm motility is considered the best biomarker for fish sperm quality. Nowadays sperm motility is determined using Computer-assisted Sperm Analysis (CASA) system which in fish has been used since the 1990s. The main advantage of using the CASA system is acquisition of objective results and repeatable data obtained. Moreover, CASA estimates a higher number of sperm kinetic parameters, including average path velocity (VAP, µm s^{-1}), curvilinear velocity (VCL, µm s^{-1}), straight-linear velocity (VSL, µm s^{-1}), progressive motile sperm (PRG, %), movement linearity (LIN = straight-linear velocity [VSL]×VCL^{-1} ×100%), wobbling index (WOB = VAP×VCL^{-1} ×100%), amplitude of lateral head displacement (ALH, µm) and beat cross frequency (BCF, Hz). Despite CASA analysis being objective and free from human errors, it should be taken under consideration that different activation solution used to sperm activation may affected sperm motility and sperm kinetic parameters of numerous fish species. Moreover the main technical problem during CASA analysis is sperm stacking to a glass surface which makes the analysis difficult and does not give real results of motility and sperm kinetic parameters. To avoid this situation bovine serum albumin (BSA) and casein are recommended to be added to an activation solution (AS), but the information on minimal effective concentration of proteins for ide (*Leuciscus idus*) sperm motility analysis are missing.

In this study, we first compare four AS i.e. Woynarovich (68 mM NaCl, 50 mM urea at pH 7.7 and osmolality of 180 mOsm kg^{-1}), Lahnsteiner (100 mM NaCl, 10 mM Tris at pH 9.0 and osmolality of 200 mOsm kg^{-1}), Kucharczyk (86 mM NaCl at pH 7.4 and osmolality of 167 mOsm kg^{-1}) and Perchec (5 mM KCl, 45 mM NaCl, 30 mM Tris at pH 8.0 and osmolality of 160 mOsm kg^{-1}) supplemented with 0.5% of BSA to ide sperm activation. Next, we select two AS i.e. Woynarovich and Perchec solutions to determine other concentrations of BSA (0,25, 1.0 and 2.0%) and casein (0.25, 0.5, 1.0 and 2.0%) addition for possibility of their use in sperm motility measurements of ide sperm. As a controls pure Woynarovich (0.0% of BSA and casein) and pure Perchec solutions (0.0% of BSA and casein) were used. It was confirmed that suplementation of BSA and casein to Woynarovich and Perchec solutions reducing ide sperm adhesion during CASA analysis and thus led to an increase sperm motility and sperm kinetic parameters. Moreover the results of the present study show that supplementation of Perchec solution with 0.5-1.0% of BSA resulted higher MOT, VCL and ALH compared to Woynarovich solution supplemented with the same BSA concentration used to ide sperm activation. On the other hand casein addition to Perchec solution at concentration of 2.0% may be also used as a ‘‘second-choice’’ protein in the CASA analysis of ide sperm.

Keywords: ide, sperm motility, sperm adhesion, albumin, casein, CASA.
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[P28]

DEREGULATED PROTEOLYSIS, RNA DEGRADATION AND DNA DAMAGE AMONG POTENTIAL CAUSES FOR LOSS OF EGG QUALITY DURING EGG AGING IN PIKEPERCH (*SANDER LUCIOPERCA*)

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A common practice in pikeperch (*Sander lucioperca*) controlled reproduction is to use a special suture technique to keep the eggs in the ovaries after ovulation and thus ease egg collection, *in vitro* fertilization process and specific crossbreeding. However, this practice leads to egg aging potentially reducing egg quality. Egg aging is particularly studied in farmed fish species for which high quality eggs are required to produce healthy offspring, however, molecular mechanisms impacted by egg aging are still poorly investigated in fishes and remains unexplored in pikeperch. This study aimed at investigating molecular processes impacted by egg aging with potential effect on pikeperch egg quality. Eggs were striped from six females at 1 and 5 hours post-ovulation (hPO) and aliquoted into 2 portions. First portion was fertilized at both time-points with pooled sperm obtained from 3 males. Fertilization (FR), hatching (HR) and deformities (DR) rates were the parameters used to assess egg quality. Second portion was snap frozen for further transcriptomic analysis using RNAseq. Egg quality was reduced at 5hPO ($p<0.05$) for all measured parameters (FR – 1hPO=89.6% and 5hPO=47.8%; HR – 1hPO=78.1% and 5hPO=23.1%; DR – 1hPO=1.6% and 5hPO=6.1%). RNAseq results also presented differences between both time-points. In total, 225 genes were differentially expressed ($p<0.05$). Gene functional analyses revealed 29 KEGG pathways ($p<0.01$) and 19 Gene Ontology Biological Processes (BP) impacted by egg aging. The most represented pathways involve signal transduction, immune system and protein and RNA degradation. Concerning BP, gene expression and chromosome organization processes were highly significantly affected while DNA damage response was the most impacted (p=2.5E-05). According to these results, eggs following ovulation should be collected earlier than 5hPO to lower the risks of drastic reduction of reproductive success. Additionally, important transcriptomic variation highlights specific metabolic changes related to egg aging. These are potentially reflected in the egg quality obtained and may help understanding the molecular mechanisms behind observed developmental failures.

Keywords: hatching, transcriptome, fertilization, deformities, mRNA

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[P29]

FIRST REPRODUCTION OF 21TH YEARS OLD RUSSIAN STURGEON'S AND LARVAL REARING

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We were brought fertilizated sturgeon eggs from the Krasnodar Research Institute of Fisheries (Russia) in 2001 to the Istanbul University, Faculty of Aquatic Sciences, Sapanca Inland Water Fish Culture Research and Applied Station. Russian sturgeon (*Acipenser gueldenstaedtii*) males obtain sexual maturity at the age of 3–4 years, while females become mature at 6–8 years. Generation intervals in farm condition 1-3 years. Male and Female Russian sturgeons reached 21 years old in ambient temperature in shaded conctare rectangular ponds (8.30 x 15 x 1.15 m). Water temperature ranges from 6 to 18 °C during the year. A female (16 kg) and two males (11 -7.8 kg) were spawned successfully. Carp pituitary hormone is prepared as 5 mg/kg for female with 2 doses; 4mg/kg for males with one dose. Gametes were got after 45 hours. One female has 2.135 kg of eggs. Tannin (0.5 g/L) was used for egg de-adhesion. The water temperature was 14-18 °C in the pond. Before the fertilization sperm motility was investigated with CASA System. The mean motility rate was 86.7%. Fertilized eggs were incubated in Mcdonalds Jars capacity of 6 L. After 5 days, fertilizated eggs hatched and then 6 days later they consumed their yolk sac. First feeding was started with *Artemia urmiana*, and then they fed with powder feed with *Artemia*. After 18 days later, all larvae started to feed with commercial feed on their diet. In conclusion, 21 years olds female sturgeon could successfully fertilize despite not spawning ever before.

Keywords: sturgeon, CASA, sperm motility, hatching rate, first reproduction

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[P30]

TWO DIFFERENT METHODS OF SPERM COLLECTION IN EUROPEAN CATFISH (*SILURUS GLANIS* L.)

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In this study, 6-9 year old catfish (*Silurus glanis* L.) two different methods were used to obtain sperm from 7 males. The first consisted in the collection of sperm by stripping (SS) and subsequent killing of males and extraction of testicular sperm from the removed testicles (CS).

The spermiation and ovulation were stimulated by carp pituitary (CP) injection at doses 5 mg/kg per body weight with temperature 22 ℃. Sperm in European catfish is always contaminated and activated by urine during sperm collection. Therefore, sperm were collected during stripping (SS) in an immobilization solution containing 200 mM NaCl, 30 mM Tris-HCl, pH 7. To remove the stickiness of the eggs, 0.5 g/l tannic acid was used for one minute after gamete activation with an exposure of 20 seconds.

Sufficient sperm volume was obtained by both methods of collection. As a result of sperm quality between CS and SS groups, fertilization success and hatching rate were calculated as a percentage. The fertilization rate was highest (81.87 ± 17.38%) in the SS group and lowest (66.18 ± 11.39%) in the CS group. With the exception of the malformation rate, the overall hatching rate was found to be 76.10 ± 12.39% and 53.83 ± 11.28% in the SS and CS groups, respectively. From the results obtained, it is clear, that it is not necessary to kill male catfish to obtain testicular sperm and to use for fertilization.

Keywords: Silurus glanis, fertilization, sperm, artificial reproduction

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[P31]

MALE EXPOSURE TO BPA IMPAIRS PRIMORDIAL GERM CELL MIGRATION IN ZEBRAFISH F1 EMBRYOS

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Primordial germinal cells (PGCs) colonize the gonads during embryo development migrating from an extraembryonic location to the genital ridge. In zebrafish, this migration is guided by a chemotactic gradient that involves a soluble factor produced by the somatic cells (Sdf1α), the expression of its receptor in the germ cells (Cxcr4b) and the corresponding decoy receptor in the somatic cells (Cxcr7b). In a previous research, we demonstrated that the exposure of zebrafish embryos to high concentrations of BPA (2000 and 4000 µg/L) dysregulated the expression of *cxcr4b* and *sdf1a*, impairing germ cell migration and gonadal colonization. Moreover, we have also showed that male exposure to BPA during spermatogenesis causes different malformations in their progeny, similar to those promoted by direct exposure during embryonic life. In the present study we are aimed to explore the PGCs gonadal colonization in embryos from males exposed to an environmental dose of BPA and their further long-term effects on the reproductive performance. Adult zebrafish males were exposed to the vehicle (0.014% of ethanol, representing control group) or 100 µg/L of BPA during two weeks. One week after the treatment had finished, males were mated with non-treated females to obtain F1 embryo. The number of PGCs was analyzed in embryos 24hpf by whole mount vasa immunostaining and the gene expression of *cxcr4b*, *cxcr7b*, and *sdf1a* was evaluated in genital ridges by RT-qPCR. At the adulthood F1 males were mated to analyze the breeding capacity. The number of PGCs colonizing the gonad was significantly reduced from an average of 12 in embryos from control males to an average of 2 in embryos from BPA-exposed males. The analysis of gene expression revealed a downregulation of *cxcr4b*, the gene encoding the receptor Sdf1α in the PGCs, revealing the inability of PGCs to react to the chemical gradient. The direct exposure of embryos promoted a similar reduction in the PGCs migration ability. Nevertheless, higher doses of BPA (2000 and 4000 µg/L) were required to promote this effect after direct exposure. In addition, at these higher doses the gradient was severely disturbed by the upregulation of *sdf1α*. These data indicate that the underlying mechanism could be different between direct or paternal exposure. The transmission of paternal exposure may rely on the genetic and/or epigenetic changes promoted in the spermatozoa, whereas changes in gene expression during direct embryo exposure can be also promoted by the BPA estrogenic effects. As it was observed after embryo exposure, the reduction in the number of founder PGCs does not further affects the fertility of males at the adulthood either.

Keywords: reproduction, endocrine disruptors, paternal exposure, gonadal colonization

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[P32]

POST-TESTICULAR SPERM MATURATION IN HOLOSTEI: IS IT SIMILAR TO STURGEONS' CASE?

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Fish speciation was accompanied by changes in the anatomy of the urogenital system. In evolutionarily modern fish group Teleostei (comprising some 30 000 species), male reproductive tracts that transport spermatozoa from the testes are fully separated from the excretory system. Thus, spermatozoa and urine are never mixed internally. However, in evolutionarily ancient groups of Chondrostei and Holostei (altogether just 37 species), the excretory and reproductive tracts are not separated. In sturgeons, sperm ducts enter the kidneys, where sperm mix with urine before being released into the environment during spawning. This sperm/urine mixing step has been found to be a requisite for the post-testicular sperm maturation, i.e., the capacity to activate motility in spawning environment. For the moment, sturgeon post-testicular sperm maturation is phenomenologically well described, while, in holosteans, functional intimacy of seminal ducts with kidney ducts, the existence and mechanism of post-testicular maturation process still need to be addressed.

The study was done using shortnose gars *Lepisosteus platostomus* (Holostei, Lepisosteidae). Three types of sperm samples were collected [sperm from testes (TS), from efferent ducts (EDS), connecting the testes with the kidneys, and from Wolffian ducts (WDS)]. The three types of sperm samples were shown to differ in sperm concentration (TS: 117.4 ± 52.1 x 10^9 spz/ml; EDS: 55.0 ± 24.1 x 10^9 spz/ml; WDS: 2.6 ± 1.9 x 10^9 spz/ml) and in seminal fluid (SF) osmolality (EDS: 320.8 ± 16.6 mOsm/kg; WDS: 149.9 ± 42.2 mOsm/kg; osmolality of SF from TS was not measured). Spermatozoa from WD were motile, while no motility was found in samples of TS and EDS. This fact suggests the existence of post-testicular sperm maturation in gars. To check this, the procedure of *in vitro* post-testicular sperm maturation was applied. After 40 min incubation of TS and EDS in SF obtained from WDS, not more than 5–10% motile spermatozoa were observed in TS samples, whereas, in contrast, in EDS samples the motility percentage was up to 70–80%. These data as well as essentially lower osmolality of SF and sperm concentration in WDS indicate that post-testicular sperm maturation occurs in gars in a way similar to that previously described in sturgeons. At the same time, experimental dyeing of urogenital ducts in gars and sturgeons revealed some differences between the two fish groups in the interconnection between sperm ducts and kidneys. It is concluded that structure of urogenital system and post-testicular sperm maturation in gars can be different from both sturgeons and teleosts.

Keywords: male urogenital system, testes, efferent ducts, in vitro maturation, sperm motility

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[P33]

IN-DEPTH PROTEOMIC ANALYSIS OF CARP SEMINAL PLASMA PROTEINS

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To date, 152 proteins have been identified in carp seminal plasma using one dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D-SDS-PAGE) combined with liquid chromatography-mass spectrometry (LC-MS). In the present study, using high-throughput LC-MS/MS-based proteomic analysis and updated *Cyprinus carpio* genome annotation (submitted to public databases on July 28, 2021), 1402 proteins were identified. Taking advantage of the large number of identified proteins in carp seminal plasma, we were able to perform comprehensive bioinformatic analysis (Ingenuity Pathway Analysis (IPA) and ShinyGo) and indicate major biological processes and physiological functions, which agree with our previous study indicating immune response, transport, metabolism, cell death and survival, cellular movement, protein synthesis and degradation as the most significant functions. Interestingly, in addition to previously identified important canonical pathways (protein ubiquitination pathway, coagulation and complement system, acute phase response signaling and FXR/RXR activation), IPA analysis revealed four additional pathways (EIF2 signaling, which mediates a translation control at endoplasmic stress; the FAT10 signaling pathway and the BAG2 signaling pathway, which are involved in protein ubiquitination and clathrin-mediated endocytosis signaling) that are the most enriched in carp seminal plasma. The latter pathway can mediate the uptake of extracellular vesicles/exosomes, the presence of which was revealed by GO annotation with a substantial number of seminal proteins linked to extracellular exosomes, agreeing with a previous study indicating the presence of exosome-associated proteins and exosomes in fish seminal plasma and suggesting their involvement in sperm maturation and spermatogenesis in fish. Interestingly, we identified in seminal plasma the same protein of unknown function from carp blood which has recently been discovered in our laboratory using mass spectrometry, and named cold acclimation protein 31 kDa (Cap31) with possible involvement in acclimation to cold temperatures and in innate immunity. In summary, we extended the tenfold list of carp seminal plasma proteins and provided a higher number of proteins associated with specific functions, biological processes and canonical pathways, which allowed a better understanding of the mechanism underlying reproductive tract physiology in carp males.

Keywords: Cyprinus carpio, fish, proteome, mass spectrometry, protein functions

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[P34]

SEAWATER PH DOES NOT AFFECT ALL THE AQUACULTURE MARINE FISH SPERM MOTILITY

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The climate change includes a decrease in seawater pH, and an increase in its temperature. It is possible that the marine fish sperm cells, which are released to the sea at spawning, can be affected by these expected changes on the water, and become unable of fulfill its role on fertilization. For that reason, different seawater pH has been tested in 3 aquaculture marine species: the European eel (*Anguilla anguilla*), the European seabass (*Dicentrarchus labrax*), and the Senegalese sole (*Solea senegalensis*), and their sperm motility parameters analyzed by a CASA-mot system.

In the European eel, tested seawater pH, from 6.5 to 9.5, affected sperm motility and other kinetic parameters like MP, FA, VCL, VAP, LIN, STR, WOB, ALH and BFC. pH values lower than 7.8 or higher than 8.2 caused lower values of motility and the rest of kinetic parameters. The longevity was not affected by pH from 7.6 to 8.2. In other experiment a seawater pH of 7.8 caused lower motility, FA, VSL, VAP and LIN than a seawater with pH 8.2. The effect of the water temperature was tested, by comparing activation with seawater at 4 °C (our control) and at 23-24 °C. The spermatozoa beating cross frequency (BCF) was the only parameter significantly affected, being lower at 23 °C than at 4 °C. In other experiment where we combined 2 temperatures (4, 24 °C) and two pH (7.8, 8.2), a significant interaction was observed, and in general the worst results were obtained with seawater at 4 °C and at pH 7.8. Considering all these data, it seems that the seawater pH has a deeper effect on the eel sperm motility than a high temperature.

Regarding European sea bass and Senegalese sole, motility and other sperm parameters were not affected by seawater temperature in the pH range from 6.5 to 9.5, thus indicating that ocean acidification would not affect their behavior. The differences found between these species, evolutively and ecologically differents, could reflect different activation mechanisms of the spermatozoa motility.

Keywords: climate change, acidification, seawater temperature, resilience

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[P35]

EXPOSURE TO SILVER AND TITANIUM DIOXIDE NANOPARTICLES DECREASED SPERM MOTILITY AND AFFECTED SPERMATOZOA SUBPOPULATIONS IN GILTHEAD SEABREAM

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Sea pollution by nanoparticles (NP) has increased in the past decades. These contaminants can be reprotoxic for fish and thus disturb successful reproduction of wild fish populations. Recent research has proven a mild effect in sperm total motility in gilthead seabream (*Sparus aurata*), after exposure to high concentrations of silver NPs, considering spermatozoa as a homogenous population [1]. However, several studies indicated that there is a great diversity in spermatozoa within a same sperm sample. It is possible that NPs toxicity affects sperm population according to their heterogeneity traits, modulating subpopulation profile. Thus, in the current work we aimed to analyse the effect of these NPs in all spermatozoa population structure and using a subpopulation approach. Gilthead seabream sperm samples (n=15-20) were collected from mature males and afterwards exposed for 1 hour to increasing concentrations of silver (0.25, 25 and 250 µg/L) and titanium dioxide (1, 10, 100, 1000 and 10000 µg/L) NPs, dissolved in a non-activating medium. After this *ex vivo* exposure, sperm motility parameters were determined using Computer Assisted Sperm Analysis, both for control and experimental groups. Sperm subpopulations were identified by applying a two-step cluster analysis. The results revealed a significant reduction in terms of total and progressive motility after exposure to all concentrations of Silver NPs, in comparison to the control. Curvilinear and straight-line velocities were significantly lower than the control only at the highest concentration (250 µg/L). Exposure to titanium dioxide NPs lowered significantly total and progressive motilities at the 3 highest concentrations (100, 1000 and 10000 µg/L), while curvilinear and straight-line velocities were not altered. Sperm subpopulations were also affected by the exposure to both Silver and titanium dioxide NPs. In both cases, the highest levels in NPs triggered a decrease on the percentage of fast sperm subpopulations, while an increase on slow sperm subpopulations. These results show how detrimental these pollutants can be for sperm motility in gilthead seabream, thus highlight the importance of monitoring their levels in the sea.

Keywords: pollutant nanoparticles, toxicity, ex vivo exposure, sperm subpopulations

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References:

[1] Carvalhais A, et al. (2022). Ex vivo exposure to titanium dioxide and silver nanoparticles mildly affect sperm of gilthead seabream (*Sparus aurata*) - A multiparameter spermiotoxicity approach. *Mar Pollut Bull.*

[P36]

INTRACELLULAR ALKALINIZATION IS NOT A UNIVERSAL FACT DURING SPERM MOTILITY ACTIVATION

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Sperm activation involves ion fluxes as well as a previous maturation in the seminal plasma, something which has not been studied in depth in marine fish species. pH and potassium are probably involved in sperm maturation and motility in the European eel (*Anguilla Anguilla*), as indicated in previous studies.

In this work, the absolute intracellular concentration of potassium in European eel sperm has been determined for the first time, being in mean 124 $\pm$ 30 mM. In addition, the intracellular pH (pH_i) of quiescent eel spermatozoa was determined by two methods (nigericin and null point) that gave similar results, 7.4-7.6. The natural pH_i range of sperm samples in the quiescent stage was 7.4-8.0, with no evident relationship with sperm motility. However, a positive linear correlation was seen between sperm motility and the pH of the diluent or extracellular pH (pH_e), as well as between the pH_i and the pH of the diluent.

The pH_i change post-activation in seawater (ASW) depended on the initial pH_e of the diluent medium. Activation with ASW induced an internal alkalinization of the cells when the sample had previously been diluted in a $pH_e < 8.0$; an acidification when $pH_e > 8$, and no pH_i variation when pH_e was 8.0. These experiments indicated that a careful selection of the diluents should be performed before measuring natural pH_i changes in sperm cells. Thus, studies on the specific seminal plasma composition of marine fish species are necessary before studying their physiology. Furthermore, our study indicates that intracellular alkalinization is not a universal fact during sperm activation.

Keywords: Potassium, sperm activation, flow cytometry, European eel

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[P37]

AN OPTIMIZED NON-ACTIVATING MEDIUM FOR SHORT-TERM STORAGE OF BARRAMUNDI (*LATES CALCARIFER*) TESTICULAR MILT

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The success of artificial fertilization lies in an ability to store and handle gametes effectively while also ensuring that the functional and structural integrity of gametes is maintained. Previous studies demonstrated successful milt storage of wild-caught barramundi (*Lates calcarifer*) using marine Ringer's solution as a non-activating medium (NAM). However, this result could not be replicated using testicular spermatozoa from captive-bred individuals. Spermatozoa lysed within 30 minutes of incubation in marine Ringer's solution. Therefore, this study aimed to optimize the composition of NAM for short-term chilled storage by characterizing and mimicking the biochemical profile of seminal plasma of captive-bred barramundi. To further understand the cause of cell lysis, we tested the effect of NAM osmolality on sperm viability after 1 h incubation at 4 °C. Spermatozoa stored in NAM adjusted at 400 mOsm showed the highest sperm viability (78.2 ± 2.6%). Thereafter, we investigated the effect of NAM pH to improve the retention of sperm motility during chilled storage. After 1 h incubation, sperm motility was negatively affected by the increase in pH and remained motile only at pH 6.5 after 24 h incubation. Biochemical analysis of NAM stocks revealed a gradual rise of pCO_2 with a pH between 7.4 and 8.5, which might have inhibited the activation of sperm motility. Therefore, the buffering agent $NaHCO_3$ was replaced by HEPES. As a result, total motility was highest at pH 7.4 (35.1 ± 3.3 %) after 24 h incubation. Finally, the effect of Na^+ and K^+ concentrations on sperm motility was tested. After 1 h incubation, sperm motility was inhibited in Na^+-free NAM. After 24 h incubation, spermatozoa in 185 mM NaCl/5 mM KCl NAM had significantly higher total motility (47.7 ± 7.2%), and progressive motility was retained for up to 72 h. These results lay the foundation for future studies looking at the regulatory role of different ions in barramundi sperm motility. More importantly, this optimized NAM facilitates safe handling and short-term storage of testicular spermatozoa for the first time, thereby facilitating the development of advanced reproductive technologies for captive-bred barramundi.

Keywords: sperm quality, biochemical analysis, chilled storage, CASA, aquaculture

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[P38]

SEPARATION OF HIGHLY MOTILE SPERMATOZOA FROM CRYOPRESERVED STERLET SPERM IS A PROMISING METHOD FOR IMPROVING OFFSPRING QUALITY

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Sperm cryopreservation is widely used in biodiversity protection and animal husbandry programs. It is well known that cryopreservation increases the risk of sperm DNA damage, which may decrease fertilizing ability and entail embryo developmental abnormalities after applying cryopreserved sperm. A specific selection (separation) of frozen-thawed spermatozoa with preserved physiological parameters (mainly motility and intact membrane) increases the chance for successful fertilization and offspring development. Nowadays, the separation of motile fractions from cryopreserved sperm is widely used in mammalian species where the number of oocytes is restricted, thus increasing the impact of each spermatozoon, which participate in fertilization. In contrast, for fish species, when thousands of eggs from one individual are involved in fertilization, only a few spermatozoa separation methods appeared in the last decade. This research aimed to determine the influence of the Percoll gradient centrifugation method on fertilization ability and offspring development of cryopreserved sperm of sterlet (*Acipenser ruthenus*).

After applying Percoll gradient separation to fresh and cryopreserved sperm with motility percentages of 74±12% and 45±20%, correspondingly, we got an increased motility percentage for fresh (81±11%) and cryopreserved sperm (76±16%), other motility parameters, such as curvilinear velocity, and linearity of spermatozoa trajectory, were not significantly different in all investigated cases. For a fertilization test, fresh sperm, cryopreserved, cryopreserved-separated, and washed from cryoprotectant after cryopreservation samples, were used. All cryopreserved sperm samples had a lower fertilization rate at the same egg/sperm ratio compared to fertilization with the fresh sample (88±16%). However, washed from cryoprotectant samples had a significantly lower fertilization rate (36±20%) than cryopreserved-separated (61±15%). Cryopreserved-separated sperm samples also had the significantly lowest percent of abnormally developed larvae (1.8±1.3%) compared to not separated cryopreserved sperm (10±1.6%). It can be concluded that Percoll gradient separation allowed a selection of motile spermatozoa, application of which for fertilization leads to offspring with low levels of developmental abnormalities.

Keywords: *percoll gradient separation, Acipenser ruthenus, offspring quality*

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[P39]

SPERM CRYOPRESERVATION OF CHUM SALMON (*ONCORHYNCHUS KETA*)

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Sperm cryopreservation is one of the options for preservation of genetic resources of fish. Glucose-methanol (GM: 180 mM glucose and 0.9% methanol) extender was widely used for sperm cryopreservation in salmonid fish. Chum salmon *Oncorhynchus keta* is anadromous and distributes in the northern Pacific. Though chum salmon is a major captured fish production in Hokkaido, the amount of captured production have been decreased dramatically recent years. Thus, the genetic resources of chum salmon need to be preserved to conserve their genetic diversity. The aim of this study was to evaluate efficacy of sperm cryopreservation in chum salmon using GM extender.

Semen samples (N = 18) were collected from mature chum salmon during the spawning season at Shibetsu Salmon Museum. The semen was diluted with GM extender at a 1:5 ratio (semen:extender) and equilibrated for 15 min on ice. Then, the diluted sperm were frozen by liquid nitrogen vapor for 5 min, and then plunged into liquid nitrogen. Sperm motilities were assessed for fresh, fresh-diluted, and post-thaw semen using CASA. Sperm concentration and seminal plasma parameters (pH, electric conductivity, DNA concentration and protein concentration) were also measured.

The sperm motility of post-thaw semen was significantly low and variable (4.2% to 64.6%) compared with those of fresh and fresh-diluted sperm. Maximum coefficient of determination between sperm motility of thawed semen and semen parameters was 0.2192 in pH of seminal plasma. In conclusion, GM extender is available for sperm cryopreservation in chum salmon, though cryopreservation success was variable among the individuals and may be related to quality of fresh semen.

Keywords: glucose-methanol extender, Salmonid, sperm motility, interoparity, semelparity

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[P40]

IN VIVO AND *IN VITRO* AGING OF COMMON CARP (*CYPRINUS CARPIO*) SPERM AFTER MULTIPLE HORMONAL APPLICATION AND STRIPPING OF MALES

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Knowledge of the sperm aging processes during short-term storage *in vivo* and *in vitro* is important for mastering successful artificial reproduction of common carp (*Cyprinus carpio* L.). The present study on common carp sperm was designed to evaluate changes in sperm phenotypic variables during multiple sperm stripping with sperm storage: a) *in vivo* and b) *in vitro*. Similar males were multiple injected with carp pituitary (CP) 3 times 3 days apart. Sperm were stored *in vivo* in the body cavity for 0.5 days (fresh sperm) and 3 days (old sperm) after CP application, then sperm were collected and diluted with a carp extender 1:1 and stored *in vitro* on ice for 0, 3 and 6 days. In general, fresh sperm from the first stripping had slightly better quality and quantity than old sperm from the second and third stripping, especially in the phenotypic parameters of number of total spermatozoa and number of total motile spermatozoa. The highest kinetic and quantitative sperm variables were obtained in fresh and old sperm just after sperm collection at 0 days and then they decreased during *in vitro* sperm storage up to 6 days. The fertilization, hatching and malformation rates from fresh sperm were similar compared with the old sperm. From the first stripping, fresh sperm, even stored for 6 days *in vitro*, showed fertility, hatching and malformations at 92.5%, 91.5% and 1.3%, respectively. Multiple hormonal treatments with multiple male stripping together with 0.5 days of *in vivo* sperm storage followed by 6 days of *in vitro* storage are methods that can be recommended for use in common carp aquaculture.

Keywords: common carp, sperm aging, sperm storage, in vivo, in vitro

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[P41]

OPTIMIZATION OF THE STORAGE MEDIUM FOR CRYOPRESERVATION OF SPOTTED WOLFFISH (*ANARHICHAS MINOR*) SPERMATOZOA

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Further development of sperm storage in an underrepresented marine cold-water fish, the spotted wolffish (*Anarhichas minor*), is crucial in the aquaculture expansion and species diversification for human consumption. The aim of this study was to assess the basic characteristics of spotted wolffish fresh sperm and optimize the experimental cryopreservation protocol currently available. Eight different novel storage media, namely, modified turbot (MT), Kime and Tveiten (KT), modified Mounib's (TS-2), ocean pout (OP), modified halibut (MH), Hank's balanced salt solution (HBSS), guppy (SR), and seawater (SW) as control were examined. Firstly, sperm samples of three individual spotted wolffish males (10 ± 1 kg body weight) were obtained by gentle stripping on disposable plastic pipettes and kept on ice for 4 h. Sperm motility, velocity, and its associated parameters (VCL, VSL, and VAP) were assessed utilizing a computer-assisted sperm analysis (CASA) system. The effectiveness of the eight extenders was evaluated at two different sperm: extender ratios (1:1 and 1:3) by maintaining the resulting diluted aliquots on 1.5 ml Eppendorf tubes at 4-8°C for 72h in a house-hold type refrigerator. KT, OP, MT, HBSS, and SR at the 1:3 ratio showed the highest sperm motility. Then, these solutions were supplemented with 10% of either one of the following permeable cryoprotectants (methanol [CH_3OH] and dimethyl sulfoxide [DMSO]) and tested as previously described. The efficiency of DMSO in combination with the five extenders tested was the most consistent. Finally, spotted wolffish fresh sperm (n=3) was diluted with each of the five extenders at 1:3 ratio containing 10% DMSO, deposited in 1.5 ml freezing straws, and cryopreserved in liquid nitrogen. The post-thaw quality of each sample was measured after 24h. The MT extender followed by HBSS and KT, respectively were the best performers. In conclusion, the present data support an optimized sperm cryopreservation protocol and provide further insight into the reproductive biology and production science of the spotted wolffish.

Keywords: CASA, cryoprotectant, dilution ratio, extender, sperm quality

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[P42]

PROTOCOL OF COMMON CARP SPERM CRYOPRESERVATION IN SAMPLES OF BIG VOLUME

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Despite a long history of cryobiological research in common carp, one of the important aquaculture species, there are still unresolved technical problems associated with the use of sperm cryopreservation for fertilization of large volumes of eggs in fisheries practice. In most cases, only one cryoprotective agent was used, much less often a mixture of two cryoprotectors. This technology successfully uses cryopreservation sperm in straws of 0.25 ml or 0.5 ml. But much more convenient is to use a bigger volume of samples for fertilization in aquaculture. The research aims to establish an optimal protocol for carp sperm cryopreservation in 4.5-ml cryotubes.

Two cryomedia were used in the study: 350 mM glucose, 30 mM TRIS, 11.1% methanol, pH 8.0 (medium 1, osmolality created by osmotically active substances 390 mOsm/kg); and 60 mM NaCl, 2.9 mM sucrose, 22% methanol, 5% ethylene glycol (medium 2, osmolality created by osmotically active substances 123 mOsm/kg). After diluting with each cryomedium, sperm samples were placed into 0.5-ml plastic straws or 4.5-ml cryotube and incubated for 10 min before freezing. Then the straws were frozen using uncontrolled cooling in a styrofoam box, with avarage cooling rate 40°C/min. The cryotubes were frozen by a programmable freezer with cooling rate 2°C/min to -20°C, 20°C/min to -180°C. Phase-contrast video microscopy technique followed by CASA was used to analyze sperm motility before and after cryopreservation. Spermatozoa volume changes after mixing with cryoprotective media were studied using a spectrophotometer equipped with a thermo-controlled chamber in a 1-cm light path cuvette.

Applying both cryomedia, it was possible to get a high post-thaw sperm motility percentage (around 50%). However, that was possible only when cryomedium 1 was used in combination with freezing in 0.5-ml straws, and cryomedium 2 was used in combination with freezing in 4.5-ml cryotubes. At the end of the incubation, spermatozoa in the cryomedium 1 were in a compressed state, while in the cryomedium 2 – in a swollen state. For the first time, we demonstrate that the success of carp sperm cryopreservation in big volumes can be achieved using slow cooling rates and cryomedium with a low level of osmotically active substances.

Keywords: Cyprinus carpio, sperm motility, cooling rate, cryomedia

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[P43]

SPERM CRYOPRESERVATION PROTOCOLS OF AMAZON SPECIES: A REVIEW

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The protocols for the cryopreservation of native South American migratory species sperm began to be investigated in the early 1980s, with a total of 127 articles published until 2022. Cryopreservation protocols have already been investigated in more than 20 South American species, 30% of which originate from the Amazon basin, representing more than 25% of the total publications. It is essential to highlight that all six Amazonian species investigated, *Colossoma macropomum*, *Piaractus brachypomus*, *Brycon amazonicus*, *Pseudoplatystoma corruscans*, *Pseudoplatystoma metaense* and *Leiarius marmoratus* are used for aquaculture. The number of studies with *C. macropomum* stands out (55% of the total number of articles produced), undoubtedly due to its relevance in Brazilian aquaculture production, approximately 300 thousand tons/year, being the second most-produced species in Brazil. Some important information has already been investigated from *C. macropomum* cryopreserved sperm, such as a) construction of the breeding program for the species from biobanks constituted for this purpose; b) the only South American species that had a genomic investigation regarding the methylation provoked by permeable cryoprotectants. Another highlight is the recent studies with *L. marmoratus* cryopreservation sperm, from 2019. This fact is related to this catfish sperm used to fertilize oocytes of the genus *Pseudoplatystoma* species in the composition of the hybrid "jundiá da Amazônia" used in Brazilian aquaculture in the Amazon region. In the practical use of Biobanks in Brazil, *C. macropomum* stands out in the construction of the genetic improvement program, and *L. marmoratus* in some fish farms as a strategy for hybrid production. Finally, the protocols for Amazonian species sperm cryopreservation need to be improved for more efficient results, from the point of view of post-thawing cell viability and the evaluation of the economic viability of creating biobanks of these species as an alternative to maintaining a large number of breeders and specimens in fish farms.

Keywords: aquaculture, biobank, Colossoma macropomum, Leiarius marmoratus

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[P44]

GAMETE STORAGE AS A TOOL FOR HELPING EX-SITU BREEDING PROGRAMS IN SEVERAL ENDANGERED LEUCISCIDS ENDEMIC FROM THE IBERIAN PENINSULA

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Populations of fish species endemic of the Iberian Peninsula have been declining over the last decades due to factors such as habitat degradation, dredging and draining processes or water quality deterioration. With the tendency for global warming in Iberia, these droughts are expected to become more intense and extended in time, increasing the pressure and the risk of local extinctions. Several types of actions (from *in situ* to *ex situ* measurements) have been applied for preserving these native fish species over the last years, but the limited knowledge about their reproductive biology makes it necessary to investigate different aspects of the reproductive cycle. The main objectives of this work were to advance knowledge concerning sperm kinetics and to develop protocols for the short- and long- term storage of gametes for improving and helping breeding programs.

Populations of different endangered leuciscid species (*Anaecypris hispanica, Iberochondrostoma lusitanicum, Achondrostoma occidentale,* and *Squalius aradensis*) were sampled during the spring of 2022 both in captive populations kept at Aquário Vasco da Gama and in wild populations from different Portuguese rivers. Sperm samples were collected, and sperm motion parameters were assessed (for the first time) for all species. Sperm kinetics differed between species in motility and velocity traits, also showing a different number of sperm subpopulations. The longevity of sperm (swimming period) was also different among species: the shortest period was obtained for the wild population of *S. aradensis* (values close to zero at 40 s), and the longest swimming period for the captive population of *I. lusitanicum* (values close to zero at 120 s).

Furthermore, different storage trials were carried out diluting the sperm in a extender solution (75 mM NaCl, 70 mM KCl, 2 mM $CaCl_2$, 1 mM, $MgSO_4$, 10 mM Hepes, pH 8) at a ratio 1:20 (sperm:extender). Sperm quality (>40% of motile cells) was kept for a maximum of four days of storage, depending on the species. In addition, different cryopreservation protocols (using DMSO, Methanol and/or egg yolk) were tested for cryobanking the spermatozoa of these threatened species. Cryopreserved samples showed significantly lower motility when compared with fresh samples, and the best results were obtained for *I. lusitanicum*, reaching 20% of motile cells after thawing using 10% of DMSO supplemented with 10% of egg yolk. This study is the first of its kind to successfully achieve gamete cryopreservation of Iberian endemic and endangered freshwater fish species, developing new and useful tools to complement the management and conservation programs.

Keywords: gamete quality, cryopreservation, chilled storage, sperm subpopulations

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[P45]

FIRST ATTEMPT ON STANDARDIZATION OF CRYOPRESERVATION PROCEDURE OF ATLANTIC STURGEON (*ACIPENSER OXYRINCHUS*) SEMEN

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Several sturgeon species, including Atlantic sturgeon are close to extinction due to stock exploitation for meat and caviar as well as habitat destruction. Sperm cryopreservation has particular meaning for gene banking of valuable strains and endangered populations to maintain genetic diversity, for synchronization of artificial reproduction and efficient utilization of semen during artificial reproduction. The standardization of the freezing and thawing process is required to develop commercial applications of sperm cryopreservation for fish. Recently, a successful attempt has been made to standardize the cryopreservation protocol using simple glucose-methanol extender for many salmonid species, however this approach has not been applied for sturgeons. The aim of this study was to develop the standardized cryopreservation procedure for Atlantic sturgeon semen through evaluation of the effects of final sperm and glucose concentrations on post-thaw sperm motility.

The sperm concentration of fresh semen (n=3) was measured using a NucleoCounter SP-100 computer-aided fluorescence microscope. Sperm motility in fresh and frozen/thawed semen was measured by CASA. The final glucose concentrations in the straw were tested at 0.025, 0.05, 0.075, 0.1, 0.15 and 0.2 M in 7.5% methanol combined with a constant final sperm concentration in the straw (1×10^9 spermatozoa ml^{-1}). Final sperm concentrations were evaluated at 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5×10^9 spermatozoa ml^{-1} combined with a constant final concentration of 0.05 M glucose at 7.5% methanol. Diluted semen was loaded into 0.5 ml plastic straws and frozen in liquid nitrogen vapour for 5 min. The straws were thawed by immersion in a water bath at 40°C for 10 s. The sperm concentration of fresh semen ranged from 1.72 to 2.10×10^9 spermatozoa ml^{-1}. Remarkably high post-thaw sperm motility was observed at 0.05 M of glucose (81±7%) and these values were significantly higher than those recorded at 0.1 and 0.2 M of glucose (54±17 and 33±15%, respectively). The highest post-thaw sperm motility was recorded at 0.75 and 1×10^9 spermatozoa ml^{-1} (82±3% and 84±5%, respectively). Our results clearly indicate that post-thaw sperm motility of Atlantic sturgeon is highly influenced by final glucose and sperm concentration. Our results demonstrated that Atlantic sturgeon semen could be successfully cryopreserved using the simple glucose-methanol extender which can be subsequently used for long-term conservation of endangered species.

Keywords: sperm motility, freezing/thawing, glucose concentration, sperm concentration

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[P46]

SUPPLEMENTATION OF EXTENDER WITH ASCORBIC ACID, TAURINE AND TOCOPHEROL DID NOT PREVENT OF THE ROS$^+$ PRODUCTION IN CRYOPRESERVED SEMEN OF SEX-REVERSED FEMALES RAINBOW TROUT

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Sex-reversed females (SRF; masculinized females, neomales) possess less-developed testes without functional spermatic ducts. Therefore, the semen of SRF resembles testicular rather than ejaculated semen and is often characterized by low or highly variable quality. Cryopreservation of fish sperm is an important tool for conservation of biodiversity, efficient and selective fertilization, and for synchronization of artificial reproduction. However, cryopreservation causes irreversible cell damage, among other, due to reactive oxygen species (ROS) production, resulting in a significant reduction in sperm quality. Damage to sperm function can be minimized by the addition of antioxidants to the extender media prior to cryopreservation. The aim of this study was to evaluate the effect of addition of three antioxidants (L-ascorbic acid - ASC, taurine - TAU and α-tocopherol - TOC) to the extender on the ROS$^+$ production in SRF rainbow trout semen after cryopreservation. The cryopreservation of semen was performed using glucose-methanol extender at a final concentrations of 0.15 M glucose, 7.5 % methanol at 3.0×10^9 spermatozoa ml^{-1} in the straw (control). Extender was supplemented with antioxidants at different concentrations: ASC and TOC – 0.1, 0.5 and 1 mM; TAU– 25, 50 and 75 mM). Diluted semen was loaded into 0.5 ml plastic straws and frozen in liquid nitrogen vapour for 5 min. The straws were thawed by immersion in a water bath at 40°C for 10 s. The reactive oxygen species in the samples were measured using a Muse Oxidative Stress Kit. Cryopreservation increased ROS$^+$ production in semen at each tested antioxidant concentrations, both compared to fresh and equilibrated semen. However, supplementation of extender with antioxidants did not result in inhibiting of ROS$^+$ production. Some differences were observed among various concentrations of antioxidants. Lower values of ROS$^+$ after freezing/thawing were recorded in extender supplemented with 0.5 mM ASC compared to 1 mM ASC. Lower values of ROS$^+$ after cryopreservation were demonstrated at 25 mM TAU than control samples and at 50 mM TAU. The highest values of ROS$^+$ were recorded in frozen/thawed (F/T) semen at 50 mM TAU. Moreover, higher values of ROS$^+$ in F/T semen were observed in extender supplemented with 1 mM TOC than in control samples and in extender supplemented with 0.5 mM TOC. In conclusion, obtained results demonstrated that for cryopreservation of semen of SRF rainbow trout, the incorporation of L-ascorbic acid, taurine and α-tocopherol at tested concentrations did not improve post-thaw sperm quality by reducing the production of ROS$^+$. Further research is needed to find effective antioxidants in counteracting oxidative stress in SRF semen during cryopreservation.

Keywords: oxidative stress, freezing/thawing, L-ascorbic acid, taurine, α-tocopherol

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[P47]

BROOK TROUT SPERM COULD BE SUCCESSFULLY CRYOPRESERVED UP TO 8 DAYS FROM ITS COLLECTION

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Long-term storage of fish semen enables the preservation of sperm from the most valuable individuals or those with good functional characteristics and allows for their subsequent use in artificial reproduction in hatchery conditions. Sperm motility is one of the most useful parameters of sperm quality and it correlates with the number of fertilized eggs. The aim of this study was to investigate the motility parameters of brook stout (*Salvelinus fontinalis*) semen stored in a liquid state (4°C) in various extenders and their influence on the cryopreservation procedure. Semen samples obtained from 4 males specimens, after dilution with 3 extenders in 1:2 ratio (artificial seminal plasma for salmonids, composition: 100 mM NaCl, 40 mM KCl, 3 mM $CaCl_2$, 1.5 mM $MgCl_2$, 20 mM Tris, pH 8.2; Cryo-KCl, composition: 150 mM trehalose, 40 mM KCl; Cryo-Tris-KCl, composition: 150 mM trehalose, 40 mM KCl, 20 mM Tris, pH 8.5) were subjected to motility analysis on the 1st, 4th and 8th day from collection using CASA system. After taking measurements on a given day, the samples were diluted in 1:3 ratio with a solution of 200mM trehalose with the addition of 10% methanol and subjected to a cryopreservation procedure. After thawing, the spermatozoa motility parameters were checked again. Along with the storage time of samples, a slight decrease in the percentage of sperm motility (MOT) was observed in the following days of storing semen in a liquid state (from 92 to 88% of motile spermatozoa). The obtained results indicate that brook trout semen stored in a liquid state is characterized by similar values of MOT in the following days of storage, regardless of the extender used. The study also showed that the cryopreservation process significantly influenced sperm motility. Among all used diluents, the highest post-thawed motility parameters were observed after the use of Cryo-Tris-KCl buffer for short-term storage of semen. In the following days of storage post-thawed motility was 76% on the 1st day and 67 and 59% on the 4th and 8th day after collection. Obtained results indicate that the semen of salmonids does not need to undergo the cryopreservation procedure immediately after collection to obtain good motility parameters after thawing. Those data also indicate that the use of an appropriate diluent can increase the survival of gametes during the freeze-thawing process.

Keywords: cryopreservation, salmonids, Salvelinus fontinalis, CASA, sperm motility, spermatozoa

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[P48]

FIRST SPERM CRYOPRESERVATION SUCCESS OF AN ENDANGERD STINGRAY: *DASYATIS HYPOSTIGMA*

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It is estimated that almost 40% of elasmobranch species are under threat of extinction because they are long-lived, slow to reproduce and are subject to by-catch and overfishing. Ex-situ conservation programs, such captive breeding, can be one of the tools used to improve the status of some of the most sensitive species belonging to this group. In that sense, the development of sperm cryopreservation techniques could help these ex-situ breeding programs, but protocols must be designed and applied in the new target species.
A species that was recently classified as endangered by International Union for Conservation of Nature (IUCN) is the groovebelly stingray, *Dasyatis hypostigma*. This species is endemic to the South Atlantic, and it lives on sand or mud bottoms in shallow coastal waters in southern Brazil, Uruguay, and Argentina. It is one of the most common rays caught in artisanal and commercial demersal trawl and gillnet fisheries and may also be negatively affected by habitat degradation and water pollution. Therefore, the main objectives of this work were to develop cryoprotocols for the long-term storage.

Sperm samples were obtained by abdominal massage from two males of *D. hypostigma,* kept in the Oceanic Aquarium (Santa Catarina, Brazil). The sperm was diluted 1:9 in elasmobranch seminal plasma extender [1] Gacía-Salinas et al 2021), and several cryopreservation protocols based on methanol (MET), dimethyl sulfoxide (DMSO), and fresh egg yolk (EY) were tested. Samples were frozen inside a box using vapor of liquid nitrogen and preserved into liquid nitrogen. The cryopreservation was carrying out using both cryotubes (2 mL) and straws (0.25 mL) to storage the cells. Sperm quality was assessed by studying the motility in fresh sperm and post-thawing samples.
The initial motility values were close to 100%. Cryopreserved samples showed significantly lower motility when compared with fresh samples, and the best post-thawing motility values were obtained with a combination of 5% DMSO and 5% methanol which showed motility values close to 35% on the cryotubes vials. For the straws, the best post-thawing motility values was over 10% and were obtained with a combination of 10% DMSO and 10% egg yolk.

This study is the first to successfully achieve gamete cryopreservation of endangered ray *Dasyatis hypostigma*, developing new and useful tool to complement the management and conservation programs.

Keywords: gamete quality, cryopreservation, conservation, Chondrichthyes

References:

[1] García-Salinas P. et al. (2021). Development of sperm cryopreservation protocols for sharks and rays: new tools for elasmobranch conservation. *Front Mar Sci.*

[P49]

SPERM SHORT-TERM STORAGE OF IDE (*LEUCISCUS IDUS*) - EFFECT OF DIFFERENT BUFFERS AND DILUTION RATIOS

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Sperm short-term storage is an old technique of stored samples of sperm in conditions of reduced metabolism (4°C). The application of this method allows preservation of a sufficient amount of sperm of the best quality for future use, i.e., fertilization, cryopreservation, hybridization or molecular studies. In practical point of view, sperm short-term storage also reduces the time-consuming and stressful manipulation with males.

In this study the effectiveness of two different buffers, i.e., Tyrode's (TLP: 100 mM NaCl; 3.1 mM KCl; 2 mM $CaCl_2$; 0.4 mM $MgCl_2$, 25 mM $NaHCO_3$, 0.3 mM NaH_2PO_4; pH 8.6) and Volckaert (VRT: 94 mM NaCl, 27 mM KCl, 50 mM glycine, 15 mM Tris–HCl, pH 7.5) on ide (*Leuciscus idus*) sperm short-term storage using a ×4 dilution ratio was analysed over 48 h. Moreover, a ×4 (1:3; sperm:buffer) compared to a ×10 (1:9; sperm:buffer) dilution ratio over 14 days of ide sperm storage using TLP buffer supplemented with antibiotics was tested. Sperm motility (MOT, %), progressively motile sperm (PRG, %), curvilinear velocity of sperm (VCL, µm s^{-1}), movement linearity (LIN, %), beat cross frequency (BCF, Hz) and amplitude of lateral head displacement (ALH, µm) were verified using CASA system. After 48 h, most CASA parameters were significantly higher in TLP in comparison to VRT buffer. It was also found that the dilution ratio has a significant impact ($P < 0.0001$) on the efficiency of ide sperm short-term storage over 14 days in comparison to undiluted sperm (Control). Sperm of ide previously diluted and short-term stored in TLP buffer supplemented with penicylin/streptomycin, regardless of the dilution ratio used, maintaining motility and fertilization capacity over 14 days at 4°C. The significantly higher fertilization rate, i.e., 70% and 73% using previously diluted × 4 and × 10 and short-term storage sperm in TLP buffer compared to fresh i.e. not subjected to short-term storage sperm (48%) was noted.

The results of the presented research may have practical application in the breeding of ide in controlled conditions, which means the possibility of using previously collected and diluted TLP sperm in egg fertilization. Moreover, knowledge about the possibility of short-term ide sperm storage under conditions of reduced metabolism may also be used to optimize the biotechnology of reproduction of other species of reophilic fish.

Keywords: ide, sperm short-term storage, dilution ratio, fertilization capacity, reproduction

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[P50]

PLOIDY ANOMALIES IN COMMON CARP (*CYPRINUS CARPIO*) PROGENY ORIGINATING FROM DIFFERENT AGED OOCYTES

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Advancing oocyte age might affect the offspring integrity. Ploidy anomalies as a form of DNA damage and the high frequency of chromosome segregation defects are associated with the ageing process. In the present study, we examined whether post-ovulatory oocyte ageing in common carp *Cyprinus carpio* is associated with the incidence of ploidy anomalies in the arising progeny at different developmental stages. Following ovulation, oocytes obtained from 6 females were stored separately at 20 °C *in vitro* for 12 h and artificially inseminated with 4 h-intervals. Ploidy levels were assessed on the 24-h embryos, post-hatched larvae, and again in 2-month-old fry fish based on flow cytometric measurements of the relative DNA content of nuclei. The incidence of ploidy anomalies in 24-h embryos developed from 0, 4, and 8-hour aged oocytes were 3%, 33% and 16% respectively. Most of the anomalies appeared as triploids while haploids, tetraploids, and mosaics were also detected. Furthermore, the ploidy anomalies in the post-hatched larvae originating from 0, 4, and 8-hour aged oocytes were 0%, 3%, and 16% respectively. The rates of ploidy anomalies decreased by the advancing of development and no anomalies was detected in 2-month-old fry fish; all examined individuals derived from different aged oocytes were diploids. Based on the obtained results, we strongly suggest detecting ploidy abnormalities before the egg hatching, as most of the embryos suffering from ploidy anomalies caused by oocyte ageing die prior to hatching and become unavailable for the calculations. The current study indicated that increasing ploidy anomalies is a pathological phenotype of post-ovulatory oocyte ageing. A detailed study of the association of fish oocyte ageing and the incidence of ploidy anomalies at the molecular level is of our interest for the future works.

Keywords: fish embryo, oocyte ageing, ploidy anomalies, post-ovulation, progeny

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[P51]

EVALUATION OF OOCYTE VIABILITY ATTRIBUTED TO POST-OVULATORY AGEING IN ZEBRAFISH (*DANIO RERIO*) USING TRYPAN BLUE STAINING

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Post-ovulatory ageing is a time-dependent deterioration of ovulated oocytes and a major limiting factor in reducing the fitness of offspring. This process may lead to the activation of cell death pathways like apoptosis, autophagy, and necroptosis in oocytes. The involvement of any possible cell death pathway in the degeneration process of post-ovulatory aged oocytes in fish has not yet been studied. In the current study, cell membrane integrity was evaluated as an indicator of oocyte viability using the trypan blue (TB) staining method. Zebrafish *Danio rerio* oocyte viability was studied during the *in vivo* ageing at 28.5 °C for 8 hours post-ovulation (HPO). The quality of oocytes was also analysed by examining their fertilizing capacity. A significant decrease in oocyte fertilizing ability and viability was observed during 8-hour *in vivo* oocyte ageing ($p < 0.05$). The values of fertilization and oocyte viability rates were 91% and 97% at 0 HPO and thereafter dropped to 71% and 89% at 4 HPO, respectively. After 8 hours of oocyte ageing, the fertilizing ability was only 3%, while 72% of the oocytes could still be recognized with the intact plasma membrane. According to the obtained results, TB staining does not accurately detect the oocyte viability loss due to the ageing. The cell death pathway might be activated in the oocytes after losing the fertilizing ability in the advanced stages of ageing. However, TB staining was useful to distinguish high- and low-quality oocytes. Low-quality oocytes exhibiting 25% of fertilization rates, showed 38% of viabilities. Thus, TB staining can be used as a simple and rapid method to estimate the quality of oocytes in zebrafish hatcheries. Complementary analyses focusing on the cell death pathways are needed for further understanding of the oocyte ageing process.

Keywords: apoptosis, cell death, fertilization, membrane integrity, zebrafish AB strain

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