9th INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

- León 2024 -

BOOK OF ABSTRACTS

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

ORGANIZERS

SCIENTIFIC COMMITTEE

Head of the Scientific Committee

- Paz HERRÁEZ, *Universidad de León (Spain)*.

Members of the International Scientific Committee

- Juan F. ASTURIANO, *Universidad Politécnica de Valencia (Spain)*.
- Julien BOBE, *National Research Institute for Agriculture, Food and the Environment INRAe (France)*.
- Elsa A. CABRITA, *Universidade do Algarve (Portugal)*.
- Oliana CARNEVALI, *Polytechnic University of Marche (Italy)*.
- Diego CRESPO, *Benchmark Genetics (Norway)*.
- Alicia FELIP, *Instituto de Acuicultura "Torre de la Sal" CSIC (Spain)*.
- Akos HORVATH, *Hungarian University of Agriculture and Life Sciences (Hungary)*.
- Catherine LABBE, *National Research Institute for Agriculture, Food and the Environment, INRAe (France)*.
- Rafael NOBREGA, *São Paulo State University UNESP (Brasil)*.
- Martin PSENICKA, *University of South Bohemia in České Budějovice (Czech Republic)*.
- Marta F. RIESCO, *Universidad de León (Spain)*.
- Vanesa ROBLES, *Universidad de León (Spain)*.
- Rüdiger SCHULZ, *Utrecht University (The Netherlands)*.
- Daniel ZARSKI, *Polish Academy of Sciences in Olsztyn (Poland)*.
- Goro YOSHIZAKI, *Tokyo University of Marine Science and Technology (Japan)*.

LOCAL COMMITTEE

Head of the Local Committee

- Vanesa ROBLES.

Members of the Local Committee

- Marta F. RIESCO
- David G. VALCARCE
- Silvia GONZÁLEZ-ROJO
- Paz HERRÁEZ
- Marta LOMBÓ

Preface

The 9th International Workshop on the Biology of Fish Gametes follows the tradition of previous excellent conferences for the scientific community interested in gametes and reproduction-related technologies of aquatic organisms. Since its first meeting, held in Vodňany (Czech Republic) in 2007, this community has gathered every 2 years in inspiring events in Valencia (Spain), Budapest (Hungary), Faro (Portugal), Ancona (Italy), Vodňany (10th anniversary event), and Rennes (France). The most recent event, in 2022, was organized by the Polish team of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences.

We are delighted to host the 9th edition of this workshop in León, Spain. This meeting serves as a crucial platform for networks and collaborations within our scientific community. We are eager to reunite with friends and colleagues and welcome new enthusiasts of fish gamete biology.

The response to the announcement of this edition has been remarkable, with the submission of numerous abstracts from scientists around the world. We have selected 42 abstracts for oral presentations, which will be presented across 6 scientific sessions: fish germ cells, preserving fish germ plasm, environmental contaminants and climate change, parental contribution, gametogenesis, and reproduction in aquaculture.

This year's program is further enriched by plenary talks from distinguished scientists, namely Dr. Goro Yoshizaki from Tokyo University of Marine Science and Technology (Japan), Dr. Oliana Carnevali from the Polytechnic University of Marche (Italy), and Dr. Francesc Piferrer from the Institute of Marine Sciences in Barcelona (Spain). We are confident that their insights will inspire all members of our unique community.

We aim to make your experience in León both productive and enjoyable.

Welcome to Spain! Let´s enjoy science together.

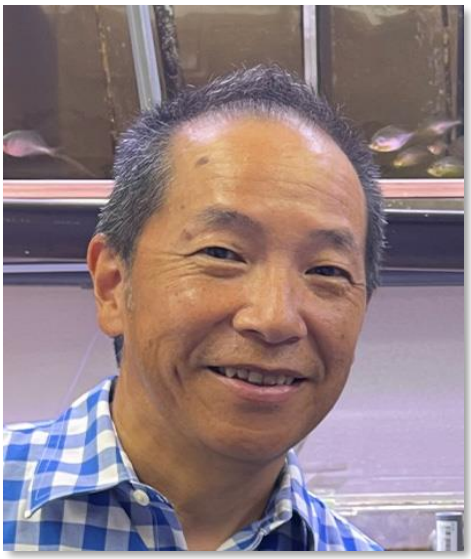
The Organizing Committee

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

KEYNOTE SPEAKERS

PROF. GORO YOSHIZAKI

Keynote speaker



Dr. Goro Yoshizaki is the current director of the Institute for Reproductive Biotechnology for Aquatic Species (IRBAS) at the Tokyo University of Marine Science and Technology (TUMSAT) (Japan). He completed his B.Sc. in Aquaculture from Tokyo University of Fisheries in 1988 and received his PhD from the same university in 1993. His graduate research focused on the development of transgenic technology using rainbow trout. He later joined Texas Tech University in the United States as a postdoctoral fellow where his research focused on elucidating the mechanisms underlying oocyte maturation in fish, amphibians, and mammals. In 1995, Dr. Yoshizaki was appointed as an assistant professor at the Tokyo University of Fisheries. He then became a professor at TUMSAT in 2012 and the director of IRBAS in 2020.

To date, he has published more than 250 peer-reviewed papers and supervised 83 master's and 21 Ph. D. students. In addition to his research on germ cell manipulation techniques, Dr. Yoshizaki is actively pursuing research on fatty acid metabolism in fish. Furthermore, he is the current president of the Japanese Society of Marine Biotechnology.

PROF. OLIANA CARNEVALI

Keynote speaker

Dr. Oliana Carnevali is Full Professor of Developmental Biology at Polytechnic University of Marche (UNIVPM, Italy). She completed her degree in Biological Sciences at the University of Camerino (UNICAM) in 1983 and received her PhD in Molecular Biology at the same university in 1989.

Her main research interests are aimed at the study of reproduction in different experimental models. This interest led her to the creation of three intertwined research pillars. The first one concerns the quality of gametes, from zebrafish to humans; an activity that has been developed within the framework of European projects or in collaboration with assisted fertilization centers. In recent years, her attention was mainly focused on Reproductive Toxicology (second pillar), in particular she has been involved in the study of reproductive disorders induced by environmental pollutants with hormones-like activities (EDCs). This scientific activity was supplemented by research efforts aimed at acquiring new knowledge on the concomitant metabolic disorders as response to contaminants in aquatic organisms, focusing on the molecular basis of such dysfunctions and on the endocannabinoid system. As third pillar she has undertaken studies on the role of intestinal microflora in well-being of organisms, reproduction, metabolism, ossification process and very recently on the role of probiotics in the mitigation of EDCs toxicity.

She has published more than 300 articles in peer-reviewed international journals and book chapters, in the majority of which she's first author or corresponding author. She has participated in 170 national and international congresses and workshops. Her H-index from SCOPUS is equal to 59 and the sum of the citations is equal to 16075 (8th January 2024).

She is Vice President of the International Society Fish Endocrinology (ISFE) (2020).

PROF. FRANCESC PIFERRER

Keynote speaker

Dr. Francesc Piferrer is Research Professor at the Institute of Marine Sciences (ICM-CSIC) in Barcelona, where he serves as deputy-director of knowledge transfer. He has worked on fish sex determination/differentiation, fish sexual systems and in developmental and environmental epigenetics. He is the author of over 160 publications that have been cited more than 13,000 times (h-index 63; Google Scholar) and has co-edited the books "Sex Control in Aquaculture" and "Epigenetics in Aquaculture" (Wiley). He is currently involved in the development of epigenetic biomarkers for application to aquaculture, fisheries and conservation biology. In 2013 he received the Jacumar prize for best research in aquaculture. In 2019 he became member of the Royal Academy of Sciences and Arts of Barcelona, and in 2020 he received the College of Biologists award for research on fish reproduction and epigenetics.

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

PROGRAM

MONDAY	TUESDAY	WEDNESDAY	THURSDAY
15.07.24	16.07.24	17.07.24	18.07.24
	*Opening (08:30–09:00 am)	Scientific Committee Meeting (08:30–09:00 am)	Session GAM Gametogenesis (08:30–11:00 am)
	Plenary session Prof. Goro Yoshizaki (09:00–10:00 am)	Keynote speaker Prof. Oliana Carnevali (09:00–10:00 am)	
	Session GERM Fish germ cells (10:00–11:00 am)	Session ENV Environmental contaminants and climate change (10:00–11:00 am)	
	Coffee break	*Coffee break*	*Coffee break*
	Session GERM Fish germ cells (11:30 am–01:00 pm)	Session ENV Environmental contaminants and climate change (11:30 am–01:00 pm)	Flash talks selected from poster sessions (11:30 am–12:30 pm)
	Lunch	*Lunch*	Session AQUA Reproduction in aquaculture (12:30–01:30 pm)
			Lunch
	Session CRYO Preserving fish germ plasm (02:30–03:30 pm)	Session QUAL Parental contribution (02:30–03:30 pm)	IMV presentation (02:45–03:00 pm)
	Coffee break and Poster session 1 (03:30–04:30 pm)	*Coffee break and* Poster session 2 (03:30–04:30 pm)	Session AQUA Reproduction in aquaculture (03:00–04:30 pm)
	Session CRYO Preserving fish germ plasm (04:30–06:00 pm)	Session QUAL Parental contribution (04:30–06:00 pm)	Keynote speaker Prof. Francesc Piferrer (04:30–05:30 pm)
			Prizes and closing (05:30–06:00 pm)
	Guided tour around historical city center (07:00–08:00 pm)		
Registration (08:00 pm)			Gala dinner (08:00 pm)
Welcome reception and registration (08:30 pm)			

*Registration desk will be open on Tuesday from 08:00 am

Session acronyms

GERM	Fish germ cells: from basic sciences to applied biotechnologies.	QUAL	Parental contribution: from gamete quality to fertilization and progeny outcome.
CRYO	Preserving fish germplasm: advances and emerging challenges.	GAM	Gametogenesis: new insights into oogenesis and spermatogenesis.
ENV	Environmental contaminants and climate change: impact on fish reproduction and sustainable aquaculture.	AQUA	Reproduction in aquaculture: managing the reproductive performance of farmed species.

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

BOOK OF ABSTRACTS

TUESDAY 16.07.24

Plenary session

[K1] Yoshizaki, G. and Morita, T. Can aquaculture be started with only male parents?

Session GERM [Chair: Dr. Goro Yoshizaki and Dr. Martin Pšenička]

[O1] Gao, L. and Pšenička, M. Replacement of mitochondria in sturgeon germline.

[O2] Nayak, R., Franěk, R., Laurent, A., and Pšenička, M. Genome-wide comparative methylation analysis reveals the fate of germ stem cells after surrogate production in teleost.

[O3] Nishimura, T. and Fujimoto, T. Induction of PGC-like cells by two germ plasm components, dnd1 and nanos3, in medaka (*Oryzias latipes*).

[O4] Kawamura, W., Ichida, K., Kamio, S., Yazawa, R., Morita, T., and Yoshizaki, G. Enrichment of donor-derived sperm produced by surrogacy using magnetic-activated cell sorting.

[O5] Lauth, X., Umazume T., Samu, S., Tsai, B., Takahashi, Y., and Buchanan, J. Partial inactivation of maternal effect gene to induce fish sterility.

[O6] Rodrigues, M. S., Chênais, N., Nóbrega, R. H., and Leyere, J-J. High resolution three-dimensional imaging and reconstruction of the whole zebrafish testis using light-sheet fluorescence microscopy (LSFM): a powerful approach to investigate the spermatogonial niche in fish.

[O7] Pšenička, M., Gao L., Nayak R., Šindelka, R., and Franěk, R. Post-ovulatory oocyte aging leads to a significant PGC decline, which affects sexual differentiation.

Session CRYO [Chair: Dr. Ákos Horváth and Dr. Elsa Cabrita]

[O8] Kodzik, N., Ciereszko A., Judycka, S., Słowińska, M., Szczepkowska, B., Świderska, B., Karol, H., and Dietrich, M. A. Cryoprotectant-induced proteomic changes in Siberian sturgeon spermatozoa following cryopreservation: Insights into the factors contributing to reduction of fertilizing ability.

[O9] França, T. S., Sanches, E. A., Teixeira, N. S., Benato, J. L., González-López, W. A., Sanchez, M. P., Ferrão, L., Fernández-García, F., Borges, L. P., Belenguer, A., Holhorea, P. G., Calduch-Giner, J. C., Felip, A., Gómez, A., Pérez-Sánchez, J., Streit Jr, D. P., and Asturiano, J. F. Biodegradable capsules as alternative cryo containers for fishes sperm.

[O10] Zhang, S. P., Shazada, N. E., Cheng, Y., Bondarenko, O., Alavi, S. M. H., Rahi, D. R., Linhartova, Z., Rodina, M., Serhii, B., and Linhart, O. Quality improvements in short-term stored sperm and its potential regulators.

[O11] Sotnikov, A., Rodina, M., Gela, D., Boryshpolets, S., Kholodnyy, V., Urbansky, A., Agemark, M., Michanek, A., and Dzyuba, B. Increase in quality of sterlet (*Acipenser ruthenus*) offspring obtained from cryopreserved samples by motile sperm fraction separation.

[O12] Nagy, B., Bokor, Z., Kaszab, E., Suhajda, Á., Farkas, M., Bartucz, T., Csorbai, B., Urbányi, B., and Bernáth, G. Detailed analysis of bacterial and fungal communities of sperm isolated from four different goldfish lines during chilled storage.

[O13] Kholodnyy, V., Dzyuba, B., Sotnikov, A., Martínez-Pastor, F., Faldyna, M., Matiašková, K., and Boryshpolets, S. Natural extracts from grape marc as supplements for fish semen extenders.

[O14] Nusbaumer, D., Karlova, Y., and Karlov, A. Advancing aquaculture and conservation: practical applications of sperm preservation technologies in fish reproduction.

WEDNESDAY 17.07.24

Plenary session

[K2] Carnevali, O. Threats of endocrine disrupting chemicals on fish gametogenesis: mechanisms and biological implications.

Session ENV [Chair: Dr. Oliana Carnevali and Dr. Rafael Nóbrega]

[O15] Giommi, C., Lombó, M., Pinto, G., Serpico, S., Illiano, A., Habibi Hamid, R., Maradonna, F. Amoresano, A., and Carnevali, O. Unraveling glyphosate toxicity on brain-gonadal axis in zebrafish female: a multiomics study.

[O16] Fedorova, G., Galicova P., Kholodnyy V., Sotnikov A., and Boryshpolets, S. From brain to sperm: effect of psychoactive drugs on male fish reproduction.

[O17] Costa, D. F., Borali, L. M., Camargo-dos-Santos, B., Bellot, M. S., Giaquinto, P. C., Habibi, H. R., and Nóbrega, R. H. Impact of bisphenols on zebrafish spermatogenesis, sperm quality, reproduction and embryo development.

[O18] Bir, J., Urrutia A., Diaz de Cerio, O., Ortiz-Zarragoitia, M., and Cancio, I. "Fish Wars" in xenoestrogenic waters: "The Ribosome Awakens" in the oocyte.

[O19] Calvo-Rodríguez, L., Ortiz-Delgado, J. B., de Paz, P., Fernández, I., and Riesco, M. F. They're here: current heat waves arrest rainbow trout (*Oncorhynchus mykiss*) spermatogenesis.

[O20] França, T. S., González-López, W. A., Fernández-García, F., Belenguer, A., Holhorea, P. G., Calduch-Giner, J. C., Felip, A., Gómez, A., Mañanós, E. L., Pérez-Sánchez, J., Pérez, L., and Asturiano, J. F. Effective challenge test to select resilient males of Mediterranean aquaculture fishes to environmental changes.

[O21] Cheng, Y., Wang, J., Alavi, S. M. H., Zhang, S., Linhartová, Z., Roy, D. R., Shazada, N. E., Dzyuba, B., Linhart, O. Impacts of global warming on fertility of male fishes: evidence from meta-analysis of temperature effects on spermatozoa motility.

Session QUAL [Chair: Dr. Catherine Labbé and Dr. Daniel Żarski]

[O22] Sandoval-Vargas, L., Pérez-Atehortúa, M., Figueroa Villalobos, E., Schulz Fontealba, F., Schulz Ferrada, M., and Valdebenito, I. The presence of inclusions in blastodiscs of coho salmon embryos (*Oncorhynchus kisutch*) is associated with low rates of fertility and embryo survival.

[O23] Depincé, A., Murat, F., Thermes, V., Brionne, A., Schartl, M., and Herpin, A. Transgenerational inheritance of DNA methylation alterations after gdf6b-/- -induced sex reversal in the medaka (*Oryzias latipes*).

[O24] Kholodnyy, V., Sotnikov, A., Dzyuba, V., Dzyuba, B., and Boryshpolets, S. Ovarian fluid affects interspecific hybridization between sterlet and Siberian sturgeon.

[O25] Rocha de Almeida, T., Schulz, P., Pajdak-Czaus, J., Nynca, J., Grecka, K., and Żarski, D. Maternal contributions to offspring immunity: beyond direct transfer.

[O26] Panda, A., Debernardis, R., Palińska-Żarska, K., Judycka, S., Klopp, C., Almeida, T. R. D., Jarmołowicz, S., Hliwa, P., and Żarski, D. Parental dispute over progeny phenotype in Eurasian perch.

[O27] Tsakogiannis, A., Marrero, C., Fatsini, E., Oliveira, C. C. V., Magalhães-Raposo, C., Rbbani, G., Fernandes, J., and Cabrita, E. Exploring new markers of gamete quality: characterization of extracellular vesicles (EVs) in fish seminal and blood plasma.

[O28] Chauvigné, F., Castro-Arnau, J., Lopez-Fortun, N., Sanchez-Chardi, A., Rutzler, M., Calamita, G., Finn, R. N., and Cerda, J. Aquaporin-3a dysfunction impairs osmoadaptation in post-activated marine fish spermatozoa.

THURSDAY 18.07.24

Session GAM [Chair: Dr. Diego Crespo and Dr. Rüdiger Schulz]

[O29] Bir, J., Diaz de Cerio, O., Ortiz-Zarragoitia, M., and Cancio, I. Differential regulation of ribogenesis genes throughout oogenesis in thicklip grey mullets (*Chelon labrosus*), preparing the maternal contribution to embryo development.

[O30] Nynca, J., Hliwa, P., Palińska-Żarska, K., Molcan, T., Lewandowski, R., and Żarski, D. Virginity's role in shaping egg quality and maternal transcript regulation in pikeperch.

[O31] Chemello, G., De Santis, L. J., Giorgini, E., Trotta, E., Maradonna, F., Carnevali, O., and Gioacchini, G. New insights into morphological and macromolecular building of spermatogenic cells of smooth-hound sharks, *Mustelus mustelus.*

[O32] Tesoriere, A., Terrin, F., Castello, I., Pagliarusco, T., Locatello, L., Martini, P., Pavlidis, M., Carnevali, O., and Dalla Valle, L. *11ß-hydroxysteroid dehydrogenase type 2* silencing impairs zebrafish male reproduction.

[O33] Ribeiro, A. O., Nakajima, R. T., Costa, D. F., Rodrigues, M. S., Marques, B. S., and Nóbrega, R. H. Single cell RNA-seq sheds light into the functional role of TGFß and BMP signaling in zebrafish germ cell populations.

[O34] López-Fortún, N., Roig, J. V., Giménez, I., Cerdà, J., and Chauvigné, F. Oxytocin and relaxin signaling pathways are involved in the gonadotropic regulation of cell-cell interaction in Senegalese sole testis.

[O35] Andersson, E., Schulz, R. W., Almeida, F., Kleppe, L., Skaftnesmo, K. O., Kjærner-Semb, E., Crespo, D., Fjelldal, P. G., Hansen, T. J., Norberg, B., Edvardsen, R. B., and Wargelius, A. Loss of Fshr prevents testicular maturation in Atlantic salmon.

Session AQUA [Chair: Dr. Juan F. Asturiano and Dr. Alicia Felip]

[O36] Zapater, C., Cruz-Castellón, C., Mazón, M. J., Morini, M., França, T. S., Pérez, L., Asturiano, J. F., and Gómez, A. Gonadotropin gene therapy in fish: from basic research to practical applications in aquaculture.

[O37] Siddique, M. A. M., Shazada, N. E., Zhang, S., Linhart, O., and Boryshpolets, S. Effects of multiple hormonal stimulation and stripping during out-of-spawning season on sperm quality of common carp *Cyprinus carpio.*

[O38] Gao, L. and Pšenička, M. The effect of visible light on early embryonic development in trout.

[O39] Oliveira, C. C. V., Marrero, C., Parente, P., Ramos-Júdez, S., Fatsini, E., Félix, F., Duarte, D., Beirão, J., Medina, D., Castro, C., and Cabrita, E. Using algae blend and micronutrients supplementation in breeders' diet to modulate gamete quality: the example of turbot.

[O40] Rashed, M. and Bondarenko, O. Mechanisms of potassium signaling in fish spermatozoa motility.

[O41] Mueni, L. M., Kholodnyy, V., Sotnikov, A., and Boryshpolets, S. Sperm motility of freshwater species- carp trout and sturgeon in viscous environments.

[O42] Erraud, A., Cornet, V., Lambert, J., Neus, Y., and Kestemont, P. Performance of offspring salmon produced using fresh and cryopreserved semen exposed to multiple stresses (thermal stress and bacterial challenge).

Plenary session

[K3] Piferrer, F. Environmental conditions during early development and their effects on the fish epigenome.

POSTER SESSIONS

Session GERM

[P1] Nayak, R., Pšenička, M., and Franěk, R. Partial gonadectomy: A new approach to obtain germline stem cells for transplantation while preserving the donor.

[P2] Blanes-García, M., Marinović, Z., Morini, M., Šćekić, I., Lujić, J., Ferrão, L., Urbányi, B., Horváth, Á., Vergnet, A., and Asturiano, J. F. Assessing recipient suitability for European eel (*Anguilla anguilla*) spermatogonia xenotransplantation.

[P3] Ferreira, J., Nóbrega, R., and Pšenička, M. Establishment of 3D testicular organoid system as a novel tool to study spermatogenesis in fish.

[P4] Balogh, R. E., Csorbai, B., Guti, Cs., Horváth, Á., Urbányi, B., Orbán, L., and Kovács, B. Dimorphic expression of sex-related genes suggests differential PGC proliferation between the two sexes of African catfish (*Clarias gariepinus*) juveniles.

[P5] Kitanović, N., Marinović, Z., Csenki, Z., and Horváth, Á. Tracking the germinal vesicle during *in vitro* maturation of transgenic *ddx4*:EGFP zebrafish oocytes.

[P6] Takeuchi, M., Fujimoto, T., and Nishimura, T. Germ cells derived from haploid embryos undergo genome doubling and generate functional gametes in medaka (*Oryzias latipes*).

[P7] Djellata, A., Zapater, C., Ibañez, S., and Gómez, A. Molecular characterization and gonad expression pattern of dead-end (*dnd*) gene in European sea bass (*Dicentrarchus labrax*).

[P8] Freitas, T. R., Marques, L. S., Lozano-Torres, K. G., Santos, R. S., Dantas, R. V., França, T. S., Albuquerque, E., Siqueira-Silva, D.H., Zhang, T., and Streit Jr., D. P. Cryopreservation of Piracanjuba ovarian tissue in biodegradable capsule.

Session CRYO

[P9] Ninhaus-Silveira, A., Sanchez, M. P., Borges, L. P., Lobato, S. I. R., Carneiro-Leite, L., Kasai, R. Y. D., Ribeiro, C. S., and Verissimo-Silveira, V. Relationship between seminal plasma composition and sperm quality parameters of the catfish *Pseudoplatystoma reticulatum*.

[P10] Figueroa Villalobos, E., Merino, O., Amorim Pereira, W., Pérez-Atehortúa, M., Niedmann, P., Avila, S., Risopatrón, J., Farías, J., Pinheiro R., Sandoval-Vargas, L., Valdebenito, I., and Villasante, A. Preliminary effects of broodstock diet composition on freezing tolerances of intratesticular spermatozoa of Atlantic salmon (*Salmo salar*).

[P11] Magny, A., Franca, T. S., García-Salinas, P., Pérez, L., Asturiano, J. F., and Gallego, V. Trying to improve cryopreservation protocols in elasmobranch sperm using new cryoprotectants and biodegradable vials.

[P12] Magny, A., Franca, T. S., García-Salinas, P., Pérez, L., Asturiano, J. F., and Gallego, V. Short-term storage in elasmobranch sperm using different pHs and temperatures.

[P13] Streit Jr., D. P., Rodrigues, R. B., Rodrigues, T. F., and Tiersch, T. Conservation of the brazilian ichitiofauna: National germoplasm bank.

[P14] Sotnikov, A., Rodina, M., Gela, D., Boryshpolets, S., Kholodnyy, V., Krasilnikova, A., Lebeda, I., Siddique M. A. M., Vazačová M., Pšenička M., and Dzyuba, B. The ploidy level of different Acipenseridae species does not influence optimal spermatozoa concentration at cryopreservation: the evidence from sperm subpopulation study.

[P15] Shazada, N. E., Zhang, S., Siddique, M. A. M., Cheng, Y., Alavi, S. M. H., Rodina, M., and Linhart, O. Optimizing extenders for short-term storage of sterlet (*Acipenser ruthenus*) sperm in hatchery condition.

Session ENV

[P16] Fernández-García, F., Carvalhais, A., Marques, A., Oliveira, I. B., Guilherme, S., Oliveira, H., Oliveira, C. C. V., Cabrita, E., Asturiano, J. F., Pacheco, M., and Mieiro, C. Short-term direct exposure to silver nanoparticles and silver ions impairs sperm motility in Pacific oysters (*Magallana gigas*).

[P17] Lombó M., Giommi, C., Maradonna, F., Pinto, G., Serpico, S., Illiano, A., Habibi, H., Amoresano, A., and Carnevali, O. Unveiling the mechanisms involved in glyphosate male reproductive toxicity.

[P18] Moraes, A. C. N. and Nóbrega, R. H. Water quality and impacts of Barra Bonita reservoir water in zebrafish spermatogenesis.

[P19] Boryshpolets, S., Kholodnyy, V., Sotnikov, A., Dyčka, F., Sterba, J., and Fedorova, G. Drugs, neurotransmitters, and fish sperm function.

[P20] Fernández-Míguez, M., Erhart, C., Yadetie, F., Karlsen, O. A., Goksøyr, A., Odei, D. K., Sørensen, L., Hosseinzadeh, M., Porte, C., and Nahrgang, J. Multi-omics assessment of gametogenesis in polar cod exposed to crude oil.

[P21] Fernández-García, F., Marques, A., Jerónimo, S., Oliveira, I. B., Carvalhais, A., Pereira, V., Asturiano, J. F., Pacheco, M., and Mieiro, C. Environmental levels of titanium dioxide nanoparticles compromise the gonads of Pacific oyster (*Magallana gigas*): gender-specific effects.

Session QUAL

[P22] Konar, E. S. M., Mai, K., Brachs, S., Waghmare, G. W., Policar, T., and Samarin, A. M. Apoptosis in oocyte aging and its impact on embryonic development in common carp *Cyprinus carpio*.

[P23] Judycka, S., Panda, A., Palińska-Żarska, K., Debernardis, R., and Żarski, D. Post-thaw storage of European perch sperm affects larval survival.

[P24] Gubanc, K., Levart, A., Kodela, T., and Sušnik Bajec, S. Fatty acid composition influences egg quality and embryo survival in grayling (*Thymallus thymallus*).

[P25] Pérez-Atehortúa, M., Sandoval-Vargas, L., Risopatrón, J., Farías, J., Figueroa Villalobos, E., and Valdebenito, I. Assessment of some egg quality parameters and chorion ultrastructure characterization in Atlantic salmon (*Salmo salar*).

[P26] Sempere, L., Marín, C., Navarro, J. C., Amaral, J., Pohlenz, C., Gómez-Requeni, P., and Felip, A. Effects of dietary macro-nutrient composition on the reproductive performance and gamete quality of European sea bass broodstock.

[P27] Dzyuba, V., Sotnikov, A., Mueni, L.M., Kholodnyy, V., Musatova, I., Policar T., and Dzyuba, B. Burbot *Lota lota* sperm contains subpopulations with different sensitivity to external C^{2+}.

Session GAM

[P28] Chemello, G., Carli, S., Giorgini, E., Trotta, E., Maradonna, F., Carnevali, O., and Gioacchini, G. Yolk composition, distribution and role in *Mustelus mustelus* a placentotrophic viviparous shark.

[P29] Abbasi, M., Janati-Idrissi, S., Nguyen, T., Thermes, V., Babiak, I. and Bobe, J. The potential role of miR-202 in reproductive phenotype of zebrafish (*Danio rerio*).

[P30] Paris, A., Ortiz-Zarragoitia, M., and Diaz de Cerio, O. Intersex gonads: searching for germ cell molecular markers.

[P31] Majewska, A. M., Dietrich, M. A., Budźko, L., Adamek, M., Figlerowicz, M., and Ciereszko, A. Identification and the potential role of Secreted Novel AID/APOBEC-like deaminase 1 (SNAD1) in carp reproductive system.

[P32] Ferrão, L., Morini, M., González-Lopéz, W. A., Gallego, V., Felip, A., Pérez, L., and Asturiano, J. F. Cold seawater pre-treatments effects on induction of early sexual maturation and sperm production in European eel.

[P33] Ferrão, L., Pérez L., Asturiano, J. F., and Morini, M. Heat shock factors in the European eel: gene characterization and expression response to different environmental conditions and to sexual maturation.

[P34] Veríssimo-Silveira, R., Quirino, P. P., Delgado, M. L. R., Gomes-Silva, L., Branco, G. S., Moreira, R. G., and Ninhaus-Silveira, A. Are androgenic steroids the agents of sexual inversion in *Brycon orbignyanus* (Bryconidae) (Valenciennes, 1849)?

Session AQUA

[P35] Martín-Montero, I. E., Rasines I., and Aguado-Giménez, F. Short-term preservation of *Chelon Labrosus* sperm.

[P36] Vidal, R., Ojeda, G., and Bañares, H. Salmonids sexing of sperm by flow cytometry.

[P37] Kodela, T., Levart, A., Horváth, A., and Sušnik Bajec, S. Effects of diet and age on the biochemical composition of grayling (*Thymallus thymallus*) eggs and gametes quality.

[P38] Horváth, Á., Jug-Dujaković, J., Kitanović, N., Balogh, R. E., Marinović Z., Špelić, I., Barić, O., and Gavrilović, A. Post-thaw survival of cryopreserved larvae of the European flat oyster (*Ostrea edulis*).

[P39] Sarih, S., Zapater, C., Gómez, A. and Felip, A. Effect of high rearing temperature on growth and gonad maturation of juvenile male European sea bass (*Dicentrarchus labrax*).

[P40] Fernández-Míguez, M., Presa, P., Puvanendran, V., Tveiten, H., Hansen, Ø. J., and Pérez, M. Gene expression and phenotypic assessment of egg quality across developmental stages of Atlantic cod.

[P41] Félix, F., Oliveira, C., Martín, I., Manchado, M., Cabrita, E., and Vera, L. M. Melatonin biosynthesis in the Senegalese sole reproductive axis.

[P42] Santos-Villangos, M., Valcarce, D. G., and Robles, V. Suboptimal culture conditions during zebrafish organogenesis do not impact on the number of *Ddx4+* cells in the genital ridge although cell migration pattern is altered.

[P43] Oliveira, C. C. V., García-Pichel, C., Marrero, C., Fatsini, E., Félix, F., Duarte, D., Parente, P., and Cabrita, E. Breeders' diet supplementation with Selenium and Zinc or Vitamins C+E modulate different sperm traits in gilthead seabream.

[P44] García-Pichel, C., Cabrita, E., and Fatsini, E. The effect of antioxidant supplemented diets and feeding regimes in sperm mRNA abundance of gilthead seabream (*Sparus aurata*).

[P45] Hernández, A., Martínez-Pastor, F., Gil, F., Sousa-Santos, C., Cabrita, E., and Gallego, V. Characterization of sperm subpopulations in two *Leuciscid* species: can the breeding in captivity affect the sperm quality?

[P46] Labesse, C., El Kamouh, M., and Labbé, C. Relevance of JC-1 for mitochondrial activity measurement in trout rainbow spermatozoa.

[P47] Sellés-Egea, A., Martínez-Vázquez, J. M., and Valcarce, D. G. Dietary probiotic supplementation from initial feeding to adulthood enhances sperm motility in the model species *Danio rerio*.

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

TUESDAY 16.07.24

Plenary session

[K1] Can aquaculture be started with only male parents?

Yoshizaki, G.[1, 2*] and Morita, T.[1]
[1]Department of Aquatic Biosciences, Tokyo University of Marine Science and Technology, Tokyo, Japan
[2]Institute for Reproductive Biotechnology for Aquatic Species, Tokyo University of Marine Science and Technology, Tokyo, Japan

*Presenting author: goro@kaiyodai.ac.jp

The first step in the aquaculture of a new fish species is the collection of eggs and sperm from the wild parental fish captured from the natural environment. We attempted to farm the highly desirable and valuable whitefin trevally (*Kaiwarinus equula*). However, the catch of this fish was extremely low. Although we managed to obtain mature males, we were unable to obtain mature females despite the efforts of many fishermen. Obviously, without the ability to collect eggs, the production of seedlings would be impossible.

To overcome this problem, we transplanted germ cells from the whitefin trevally into the hatchlings of horse mackerel (*Trachurus japonicus*), a related species within the same *Carangidae* family for which aquaculture methods are well established. However, this approach did not result in the production of gametes derived from the donor, likely due to the significant genetic distance between the donor and recipient species. Therefore, we subsequently produced hybrid F1s by fertilizing horse mackerel eggs with sperm from mature whitefin trevally males. The resulting F1 hybrids were viable but completely infertile.

We transplanted the whitefin trevally germ cells into the hatchlings of these hybrids and raised them to full maturity. This process resulted in the recipient individuals producing large quantities of donor-derived eggs and sperm. The fertilization of these eggs with sperm allowed us to obtain a substantial number of healthy whitefin trevally seedlings. Thus, we have shown that aquaculture can be started with only male parents. Notably, the germ cells used for transplantation could also be isolated from dead fish. In particular, if the germ cells were retrieved from ice-cold stored fish within one day of death, the transplanted cells were efficiently incorporated into the recipients' gonads, initiating gametogenesis.

By expanding these techniques, we hope to facilitate the aquaculture of fish species for which parent fish are currently difficult to obtain. In some cases, it may even allow aquaculture to be started even if live fish are unavailable.

Session GERM [Chair: Dr. Goro Yoshizaki and Dr. Martin Pšenička]

[O1] Replacement of mitochondria in sturgeon germline

Gao, L.* and Pšenička, M.
Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic

Presenting author: lgao@frov.jcu.cz | *Student*

INTRODUCTION

Sturgeon is one of the most critically endangered species in the world mainly due to overfishing for caviar, it is urgent and necessary to preserve the remaining diversity and aid in the recovery of this endangered species. Cryopreservation is a method for storage of living cells for a virtually infinite period of time, and the cryopreservation of sperm has been successful in several aquatic species, but we still lack the technology to cryopreserve eggs and embryos, which leads to the fact that the maternal genetics cannot be successfully preserved. That means we urgently need to establish a new technology to conserve sturgeon maternal genetics – mitochondrial DNA. The aim of the present research is to implement the replacement of mitochondria in sturgeon embryo germline. We will take advantageous of specific primordial germ cell (PGCs) development in vegetal pole of sturgeon embryo, which is isolated from other cell line precursors.

METHODS

Mitochondria were isolated from eggs of donor sturgeon, then the host embryos underwent irradiation of vegetal pole with UV to eliminate the endogenous mitochondria of germplasm. The previously isolated mitochondria were injected into the vegetal pole of the embryo at the 1-4 cell stage allowing the mitochondria to colonize PGCs. A control group was injected with FITC-dextran, which labels PGCs. To test whether PGCs development was disturb after UV-irradiation, we injected FITC-dextran into UV-irradiated embryos. To test whether mitochondria can rescue the PGCs, we injected FITC-dextran with PKH26 labelled or unlabeled mitochondria into UV-irradiated fertilized embryos. Finally, the numbers of PGCs in each experimental group were counted. The transplanted PGCs were identified by molecular and histological methods.

RESULTS & DISCUSSION

We found that UV irradiation can specifically and efficiently destroy PGCs and that injection of mitochondria from donor eggs (of the same or different sturgeon species) into UV-treated embryos rescued PGCs and restored them to the original numbers, the higher number of mitochondria injected, the higher number of PGCs rescued. We also found that mitochondria labeled with PKH26 rescued PGCs in the same manner as unlabeled mitochondria and can be successfully tracked in vivo. In addition, the mitochondria transplanted between different sturgeon species were detected using mtDNA specific primers in larvae. The preliminary results clearly demonstrate that the eliminated mitochondria can be replaced by interspecific transplantation.

[O2] Genome-wide comparative methylation analysis reveals the fate of germ stem cells after surrogate production in teleost

Nayak, R.[1*], Franěk, R.[1], Laurent, A.[2], and Pšenička, M[1]
[1]Faculty of Fisheries and Protection of Waters, USB, Vodnany, Czech Republic
[2]Fish Physiology and Genomics Laboratory, INRAE, Campus de Beaulieu, 35000, Rennes, France

*Presenting author: rnayak@frov.jcu.cz | *Student*

Spermatogonial stem cell (SSC) transplantation is the tool to produce donor-derived gametes in fish via a surrogate parent. The isolated SSCs are transplanted into the sterile recipient, where only a few cells survive and colonize the recipient's gonad. The proliferation and differentiation of the donor SSCs occur in a foreign environment. As known, epigenetics controls cell differentiation and gene expression in living organisms, which may change due to environmental intervention. In this study, we have investigated the epimutation in donor-derived sperm and their intergenerational effect. We have performed inter- and intraspecific transplantation using vas::EGFP (Tg(ddx4:egfp) zebrafish as the donor, AB zebrafish, and pearl danio as the host. For interspecific transplantation, the recipient was prepared by hybridization by crossing zebrafish and pearl danio, and for intraspecific, dead-end knockdown recipients were used. Sperm and progeny from the donor and the germline chimera were sequenced using whole-genome bisulfite sequencing to study the DNA methylation at the single-base resolution. We observed genome-wide hypermethylation in inter- and intra-specific chimera compared to the donor. Hypermethylation in the promoter regions of the protocadherin gamma gene in the intraspecific surrogates was found to be associated with germline transmission. We found that MAPK/p53 pathway disruption in interspecific surrogates due to hypermethylation in the multiple promoter regions of several genes led to inefficient removal of meiotic-arrested endogenous germ cells and production of recipient derived infertile spermatozoa. Our study provides a deeper insight into the reliability of fish surrogacy in basic science research and aquaculture.

[O3] Induction of PGC-like cells by two germ plasm components, *dnd1* and *nanos3*, in medaka (*Oryzias latipes*)

Nishimura, T.[1*] and Fujimoto, T.[1]
[1]Faculty of Fisheries Science, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido, Japan

*Presenting author: tnishi@fish.hokudai.ac.jp

In teleost fish, primordial germ cells (PGCs), origin of sperm and eggs, develop from cells inheriting maternal germ plasm accumulated in eggs. Although many component genes of germplasm have been identified such as *vasa*, *dazl*, *piwi*, *tdrd*, *dnd1* and *nanos3*, it is still unknown which components are sufficient to form PGCs in teleost fish. Here, we provide the evidence that two factors, *dnd1* and *nanos3*, have potential to induce primordial germ-like cells (iPGC) from blastomeres of medaka embryos.

Medaka embryos with overexpression of *dnd1* and *nanos3* mRNA (DN-OE) resulted in developmental arrest before gastrulation and upregulation of PGC reporter, EGFP-*nanos3* 3'UTR, in whole blastomeres. When DN-OE blastomere at blastula stage were transplanted into germ cell-deficient embryos, very surprisingly, almost all transplanted cells migrated to the gonadal ridge, resulting in gonads with huge number of germ cells. The rate of the germline chimera was 100%, which was significantly higher than that generated by transplanting uninjected blastomeres (45%). The germline chimera developed normally into females and males and produced functional eggs and sperm, respectively.

To generate medaka embryos with iPGCs without the cell transplantation, we injected *dnd1* and *nanos3* mRNA into one blastomere of 16-cell stage embryos in which endogenous *dnd1* and *nanos3* were depleted by knockdown. These embryos developed normally and possessed plenty of iPGCs expressing germline genes such as *vasa*, *piwil1*, *piwil2*, *dazl* and *tdrd1*. To ask whether the iPGCs are able to be genetically modified by genome editing, we targeted vasa locus for integration of EGFP in iPGCs and successfully obtained transgenic knock-in medaka with precise integration of EGFP at the C-terminal of VASA protein (VASA:EGFP). Immunohistochemical staining revealed that VASA:EGFP signals were colocalized with endogenous VASA protein. Altogether, iPGCs induced by *dnd1* and *nanos3* can differentiate into functional sperm and eggs, which are available for genetic modification by genome editing. In addition, DN-OE embryos have a great potential for generation of germline chimera and application for surrogate brood stock technique.

Funding: This work was supported by JST FOREST (Fusion Oriented REsearch for disruptive Science and Technology Program), Grant Number JPMJFR210D.

[O4] Enrichment of donor-derived sperm produced by surrogacy using magnetic-activated cell sorting

Kawamura, W.[1,2*], Ichida, K.[1], Kamio, S.[3], Yazawa, R.[1,3], Morita, T.[1], and Yoshizaki, G.[1,3]

[1]Institute for Reproductive Biotechnology for Aquatic Species, Tokyo University of Marine Science and Technology, Tokyo, Japan
[2]Department of Biological Sciences, Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan
[3]Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo, Japan

*Presenting author: wkawam@kumamoto-u.ac.jp

In our recent study, we demonstrated that xenogeneic germ cell transplantation enables the surrogate production of functional Pacific bluefin tuna (PBT) sperms in hybrid little tuna (HLT) of the genus *Euthynnus,* which attain sexual maturity at a smaller body size and shorter generation time. However, the semen of HLT recipients contained donor-derived PBT sperms and a large number of own sperms. Therefore, enrichment of donor-derived PBT sperm from HLT semen is required to enhance fertilization and subsequent offspring production efficiency. In this study, we developed a technique for enriching PBT sperm using magneticactivated cell sorting (MACS) and a novel monoclonal antibody that specifically recognizes live PBT sperm. First, we produced monoclonal antibodies by directly inoculating live PBT sperm into mice, which were then screened using cell-based enzyme-linked immunosorbent assay and immunocytochemistry (ICC), yielding one antibody (no. 102B) from a pool of 907. ICC analysis revealed that antibody no. 102B strongly and specifically recognizes PBT sperm but not HLT sperm. Notably, antibody-bound PBT sperm had a motility rate of 44.9% ± 2.7% (n = 3), with no significant difference observed between antibody-bound and non-bound PBT sperm. Next, we conducted MACS using antibody no. 102B on a mixture of PBT and HLT sperm at a ratio of 1:9 (10% PBT and 90% HLT sperm). Consequently, the rate of PBT sperm in the antibody-positive fraction was 56.8% ± 4.7% (n = 3), showing that MACS-enriched PBT sperm by 5.6 times over the mixed sample. Additionally, the motility rate of PBT sperm in the antibody-positive fraction was 29% ± 5.2%, indicating that MACS-enriched PBT sperm have sufficient motility for fertilization. In conclusion, antibody no. 102B, which was developed in this study specifically recognizes PBT sperm not HLT sperm, and its binding does not inhibit PBT sperm motility. Furthermore, MACS with antibody no. 102B can significantly enrich PBT sperm while maintaining motility. Hence, MACS enrichment would allow for the selective enrichment of donor-derived PBT sperm from actual HLT recipient semen, thereby increasing the production efficiency of donor-derived PBT offspring. This methodology, which combines shortened generation time through xenogeneic germ cell transplantation with MACS enrichment of donor-derived sperm, would be an effective strategy to accelerate breeding in PBT.

[O5] Partial inactivation of maternal effect gene to induce fish sterility

Lauth, X.[1*], Umazume T.[1], Samu, S.[1], Tsai, B.[1], Takahashi, Y.[2], and Buchanan, J.[1]
[1]The Center for Aquaculture Technologies, San Diego, California (USA)
[2]Department of Microbiology and Molecular Genetics, University of California Irvine (USA)

*Presenting author: xlauth@aquatechcenter.com

Targeting primordial germ cells (PGCs), the precursors of sperm and eggs in finfish, offers a promising avenue for population control and genetic management in both aquaculture and wild fisheries. Disrupting the mechanisms that guide PGC specification can lead to their loss, ultimately yielding sterile fish. By breeding fertile females carrying mutations that hinder PGC formation, we have the potential to establish sterile fish populations, thereby mitigating the risk of introducing commercially beneficial genetic alterations into natural gene pools.

The development of PGCs relies on various post-transcriptional regulatory mechanisms. Maternal mRNAs must be synthesized, packaged into oocytes, silenced, activated at precise times, transferred to PGCs, and eventually cleared from somatic embryonic cells once zygotic transcription commences. While identifying genes crucial for PGC formation, we encountered a challenge: null alleles of essential genes exhibited pleiotropic effects, hindering the fertility of homozygous mutant females.

To address this challenge, we propose selectively deactivating maternal gene function related to PGC development while preserving its zygotic activity, such as oogenesis. This partial gene inactivation involves disrupting regulatory motifs essential for post-transcriptional regulation during early embryonic development but non-essential for later stages. We demonstrated the feasibility of this approach in tilapia by disrupting the evolutionarily conserved motif1 within the 3'UTR of the nanos3 gene, resulting in females with functional ovaries but producing embryos depleted of PGCs.

Given that multiple cis-regulatory motifs likely contribute to post-transcriptional regulation of germ plasm genes, we are simultaneously disrupting several conserved motifs to achieve multiplexed precise changes in the tilapia genome. We have developed a robust CRISPR/Cas9 methodology capable of editing DNA sequences at multiple sites simultaneously along the 3'UTR of genes essential for PGC formation. Initial results indicate that the new mutant alleles do not impair adult germ cell development, offering the potential for potent maternal effect sterility.

Funding: USDA-NIFA Award No.: 2022-33522-37836.

[O6] High resolution three-dimensional imaging and reconstruction of the whole zebrafish testis using light-sheet fluorescence microscopy (LSFM): a powerful approach to investigate the spermatogonial niche in fish

Rodrigues, M. S.[1], Chênais, N.[2], Nóbrega, R. H.[1*], and Lareyre, J-J.[2]
[1]Reproductive and Molecular Biology Group, Department of Structural and Functional Biology, Institute of Bioscience, São Paulo State University (UNESP), Botucatu, Brazil
[2]INRAE, UR1037 Laboratoire de Physiologie et Génomique des Poissons, Campus de Beaulieu, Rennes, France

*Presenting author: rafael.nobrega@unesp.br

The cellular dynamic and the underlying molecular mechanisms allowing the renewal of the germ stem cell stock in adult testes remains poorly understood in vertebrates, and particularly in fish. In absence of strictly specific marker for adult germ stem cells, the identification and enumeration of the latter into the testis relies mainly on histological analyses using morphological, infrastructural and topological criteria. Despite the use of serial sections, the difficulty to distinguish on 2D images between single germ stem cells and cells forming doublets of undifferentiated spermatogonial cells is a source of inaccuracy. In addition, such a laborious approach is generally restricted to limited areas of the testis that may not reflect the overall tissue. High resolution three-dimensional (3D) imaging of whole organs is a promising technique that could circumvent caveats of the 2D histological approach. In the present study, we tested and improved a modified CUBIC tissue clearing technique to image the testis of adult transgenic zebrafish (Dr_gsdf:eGFP/vasa:DsRed) expressing the red fluorescent protein (Dsred) in spermatogonial subpopulations (including single germ stem cells) and the green fluorescent protein (enhanced GFP) in Sertoli cells. Note that the zebrafish reporter line was preferred over other cell labelling technique to ensure homogenous labelling of the targeted cells throughout the depth of the tissue. Testes were first dissected out and then rapidly fixed in 4% PFA for 1 hour at room temperature. The tissue samples were incubated in modified CUBIC 1A and CUBIC 2 clearing solutions (Clear, Unobstructed Brain Imaging Cocktail) with a nuclear DNA labelling in between using Hoechst 33342. The endogenous fluorescence and the nuclear DNA labelling were preserved throughout the testes, and were imaged deeply with recordings every 0.5 μm. 3D projections of the image stacks showed that Sertoli cells were present in all regions lining the tubular walls and delineating different cysts, while spermatogonia were either single or organized in clusters containing 2 to 16 cells. The chromatin compaction and the averaged nuclear size were decreased in the spermatogonia of 16-cells cysts. High resolution 3D imagery described in the present study is a great improvement compared to our anterior achievements (10.1038/srep43012). The improvement on image quality in depth is a prerequisite for the next step of automatic enumeration of different spermatogonial subpopulations either in vivo or in vitro 3D culture models (explant cultures, organoids).

Funding: The present study was funded by the EU-FP7 AQUAEXCEL3.0 project (871108) and the São Paulo Research Foundation (research grants: 2019/22997-3; 2022/13097-1 and 2021/06742-5).

[O7] Post-ovulatory oocyte aging leads to a significant PGC decline, which affects sexual differentiation

Pšenička, M.[1*], Gao L.[1], Nayak R.[1], Šindelka, R.[2], and Franěk, R.[1]
[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[2]Laboratory of Gene Expression, Institute of Biotechnology, BIOCEV, Vestec, Czech Republic

*Presenting author: psenicka@frov.jcu.cz

Post-ovulatory oocyte aging in fish leads to reduced fertilization and survival rates, as well as offspring abnormalities, primarily due to oxidative stress. Primordial germ cells (PGCs), crucial for sexual differentiation, are highly sensitive to oxidative stress. This study investigated the effects of oocyte aging on PGC numbers, underlying causes of PGC decline, and subsequent impact on sex differentiation. Sperm and eggs from vas::gfp transgenic zebrafish were collected and fertilized either immediately or after a 2-hour delay post-striping. Fertilization, survival rates, and PGC numbers were evaluated, alongside qPCR for PGC-specific vasa gene expression. A portion of the eggs was collected before fertilization for reactive oxygen species (ROS) assessment. Embryos were reared to adulthood to assess lifelong consequences of oocyte aging on sex differentiation. Additionally, yolk stream dynamics were traced using rhodamine-dextran and dnd1 mRNA transfer. Results showed that aged eggs had significantly lower fertilization (57.8% vs. 70.8%) and hatching rates (34.4% vs. 55.6%), and a marked decline in PGCs (12.9 vs. 27.6). The female/male ratio shifted from 49.0% to 35.1%, correlating with reduced PGC numbers. Yolk stream functionality and dnd1 transcript migration were impaired in aged eggs, linked to a 37.7% increase in ROS. Similar patterns were observed in carp and sturgeon, although sturgeon showed a less dramatic PGC decline probably due to holoblastic cleavage. This study highlights the detrimental impact of oocyte aging on PGC numbers and sex differentiation, primarily driven by elevated ROS and disrupted yolk streams.

Session CRYO [Chair: Dr. Ákos Horváth and Dr. Elsa Cabrita]

[O8] Cryoprotectant-induced proteomic changes in Siberian sturgeon spermatozoa following cryopreservation: Insights into the factors contributing to reduction of fertilizing ability

Kodzik, N.[1], Ciereszko A.[1], Judycka, S.[1], Słowińska, M.[1], Szczepkowska, B.[2], Świderska, B.[3], Karol, H.[1], and Dietrich, M. A[1*]

[1]Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland
[2]Department of Sturgeon Fish Breeding, National Inland Fisheries Research Institute, Olsztyn, Poland
[3]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

*Presenting author: m.dietrich@pan.olsztyn.pl

Semen cryopreservation is essential for preserving genetic diversity and reproductive success in endangered species such as Siberian sturgeon. Nonetheless, this process can induce cryodamage, impacting the quality and protein profile of spermatozoa. While cryoprotectants like dimethyl sulfoxide (DMSO) and methanol (MeOH) facilitate post-thaw motility and viability, DMSO-preserved sturgeon spermatozoa exhibit reduced fertilizing ability. For the first time, two complementary approaches of quantitative proteomics, liquid chromatography-mass spectrometry (LC-MS) and two-dimensional difference in gel electrophoresis (2D-DIGE), were used to analyze the proteomic profiles of fresh and cryopreserved spermatozoa as well as the extracellular medium (EM; n=7 for each group). The presence of seven proteins was verified through western blot analysis. Our results revealed that cryopreservation led to a decline in sperm motility parameters (MOT, VCL, PROG) and viability, as well as an increase in ROS levels, membrane fluidity, and acrosome damage. Despite similar values of sperm quality parameters between DMSO and MeOH-cryopreserved sperm, DMSO-cryopreserved sperm showed dramatically lower fertilization success compared to MeOH (6.2% vs 51.2%, for DMSO and MeOH, respectively). A total of 224 and 118 differentially abundant proteins were identified in spermatozoa cryopreserved with MeOH and DMSO, respectively, compared to fresh semen. Additionally, 342 and 363 proteins were released into the EM from the MeOH and DMSO-preserved spermatozoa, respectively. Moreover, cryopreservation caused alterations in the proacrosin/acrosin system within the seminal plasma. 36 and 39 uniquely altered sperm-leakage proteins were identified for MeOH and DMSO cryopreserved samples, respectively. The functional analysis of MeOH-specific proteins indicated their involvement in chromosomal structure and mitochondrial functionality, while DMSO-specific proteins were associated with acrosome reaction, zona pellucida binding, flagella structure, and nuclear pore organization. Our results indicated that loss of fertilizing ability of DMSO-cryopreserved sturgeon spermatozoa is not related to impairment of sperm motility but alteration of proteins involved in acrosome reaction, egg recognition and early stages of embryo development. This work represents the first in-depth proteomic characterization of Siberian sturgeon spermatozoa after cryopreservation with DMSO and MeOH, revealing insights into proteomic changes that affect fertilizing ability and aiding conservation efforts for this endangered species.

Funding: This work was supported by the National Science Centre (project 2019/35/B/NZ9/03501).

[O9] Biodegradable capsules as alternative cryo containers for fishes sperm

França, T. S.[1*], Sanches, E. A.[2], Teixeira, N. S.[3], Benato, J. L.[3], González-López, W. A.[1], Sanchez, M. P.[1], Ferrão, L.[1], Fernández-García, F.[1], Borges, L. P.[1], Belenguer, A.[4], Holhorea, P. G.[4], Calduch-Giner, J. C.[4], Felip, A.[4], Gómez, A.[4], Pérez-Sánchez, J.[4], Streit Jr, D. P.[3], and Asturiano, J. F.[1]

[1]Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain
[2]Fishery Engineering Course and Aquaculture Centre (CAUNESP), São Paulo State University, Registro, Brazil
[3]Aquam Research Group, Animal Science Research Program, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[4]Instituto de Acuicultura Torre de la Sal (IATS, CSIC), Ribera de Cabanes, Castellón, Spain

*Presenting author: thalesfranca@gmail.com

We aimed to evaluate the efficiency of hard-gelatin and hard-hydroxypropyl methylcellulose (HPMC) capsules as biodegradable alternative containers to plastic straws for the sperm cryopreservation of South American silver catfish (*Rhamdia quelen*, n=12), European eel (*Anguilla anguilla,* n=12), gilthead seabream (*Sparus aurata,* n=12), and European sea bass (*Dicentrarchus labrax,* n=10). Sperm samples with motility over 60% were cryopreserved in plastic straws, hard-gelatin, and HPMC biodegradable capsules. Total motility (MOT - %), curvilinear (VCL - μm/s), straight line (VSL - μm/s), and average path (VAP - μm/s) velocities were evaluated by CASA-Mot software. Rates of fertilization, hatching and normal larvae morphology were determined using post-thaw silver catfish sperm. Silver catfish sperm cryopreserved in the three containers had the same reproductive capacity and normal larvae production. To quantify DNA damage of eel, gilthead seabream, and sea bass, the alkaline comet assay was performed. Tail-DNA (%) was evaluated by CaspLab software. In all four species, sperm cryopreservation in all containers reduced MOT. However, the MOT results obtained from samples stored in capsules and straws did not differ. The same was observed for sperm velocities. However, the eel sperm samples cryopreserved in straws maintained their velocities in comparison with fresh samples, and the gelatin capsules maintained their VSL and VAP after cryopreservation. Sea bass samples stored in gelatin capsules showed higher velocities than sperm cryopreserved in HPMC capsules. Cryopreservation did not cause DNA damage in eel and sea bass sperm samples. On the other hand, gilthead seabream samples cryopreserved in gelatin (10±2%) and HPMC (11±6%) showed higher Tail DNA after thawing than fresh (4±3%) and straw (4±1%) samples. The hard-gelatin and HPMC biodegradable capsules can be considered an alternative to plastic straws for silver catfish, European eel, gilthead seabream, and European sea bass sperm cryopreservation.

Funding: MCIN NextGenerationEU (PRTR-C17.I1), GVA (THINKINAZUL/2021-012, -024, -042), MIU (RD 289/2021. UAB), GVA (GRISOLIAP/2020/063), CNPq (141717/2019-0, 200285/2021-1, 200245/2022-3), FAPERGS (17/2551-0000917-7), CAPES (88882346090/2015).

[O10] Quality improvements in short-term stored sperm and its potential regulators

Zhang, S. P.[1*], Shazada, N. E.[1], Cheng, Y.[1], Bondarenko, O.[1], Alavi, S. M. H.[2], Rahi, D. R.[1], Linhartova, Z.[1], Rodina, M.[1], Serhii, B.[1], and Linhart, O.[1]

*Presenting author: szhang@frov.jcu.cz | *Student*

[1]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Zatisi 728/II, 389 25 Vodnany, Czech Republic
[2]School of Biology, College of Science, University of Tehran, Tehran 14176-14411, Iran

Short-term storage of sperm in an extender at low temperatures is widely utilized in hatchery management. This study investigated the effects of temperature elevation on spermatozoa quality after short-term storage at 0-2°C and identified potential regulatory mechanisms. Sperm from common carp (*Cyprinus carpio* L.) was diluted with an extender at a 1:1 ratio (v/v), stored for 6-14 days at 0-2°C under aerobic conditions. Post-storage, the sperm suspension was incubated at 20°C for 0, 10, and 20 minutes, with subsequent evaluation of spermatozoa motility, velocity, viability, and fertilizing ability. Significant increases in spermatozoa motility were observed in sperm stored for 6 days following incubation at 20°C for 10 and 20 minutes and activation in both distilled water and saline solution. For sperm stored for 8-9 days, fertilization and hatching rates markedly improved after incubation at 20°C for 10 minutes (≤ 200 µL of diluted sperm) to 2 hours (≥ 6 mL diluted sperm) with activation in distilled water. Interestingly, the enhancement of spermatozoa motility with increased temperature was not linked to ATP content. However, demembranated spermatozoa demonstrated full motility initiation. Additionally, spermatozoa swelling was influenced by the temperature of the activation medium. This study offers novel insights into the temperature regulation of motility in both fresh and aged spermatozoa in fish, providing valuable information for developing strategies to enhance sperm motility and improve artificial reproduction techniques in aquaculture.

Funding: Ministry of Education, Youth and Sport of the Czech Republic (LM2023038), Czech Science Foundation (23-06426S), National Agency for Agriculture Research (QK21010141).

[O11] Increase in quality of sterlet (*Acipenser ruthenus*) offspring obtained from cryopreserved samples by motile sperm fraction separation

Sotnikov, A.[1*], Rodina, M.[1], Gela, D.[1], Boryshpolets, S.[1], Kholodnyy, V.[1], Urbansky, A.[2], Agemark, M.[2], Michanek, A.[2], and Dzyuba, B.[1]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[2]AcouSort AB, Medicon Village, 223 81 Lund, Sweden

*Presenting author: asotnikov@frov.jcu.cz | *Student*

Sperm cryopreservation is a crucial technique for preserving fish biodiversity. However, cryopreservation often results in cell death and damage. In mammals, the adverse effects of cryopreservation on spermatozoa can be mitigated by using various sperm separation techniques before artificial fertilization. Unfortunately, many of these techniques do not apply to fish due to significant physiological differences and varying motility durations between mammals and fishes. Sturgeon sperm, in particular, presents additional challenges as the acrosome can be damaged or activated during separation, causing failed fertilization. This study compares the effects of two sperm separation techniques—Percoll gradient separation and microfluidic acoustic separation—on cryopreserved sterlet sperm. The Percoll gradient method has been used for decades in mammalian research and has succeeded in several fish species. Microfluidic acoustic separation is a newer method recently adapted for sperm separation. Both techniques were found to be suitable for sturgeon sperm, succeeding in obtaining subpopulations of cryopreserved sperm with a significantly higher percentage of motile cells compared to the pre-treated sample. Fertilization tests indicated that both separation techniques did not alter the fertilization rate of cryopreserved sperm. Nevertheless, separated and non-separated samples showed a significantly lower fertilization rate than the fresh control samples. However, both separation methods significantly decreased the percentage of abnormally developed larvae compared to using whole-thawed samples. These results suggest that both separation methods effectively reduce the number of spermatozoa with damaged or altered genetic material in separated subpopulations. Nonetheless, they probably can not remove the sperm with damaged acrosomes or other cell components involved in the transfer of genetic information to the egg, which can reduce the overall fertilization capacity of the sample.

Funding: Czech Science Foundation (No. GACR 22-14069S), Ministry of Education, Youth, and Sports of the Czech Republic (project "CENAKVA ", LM2018099).

[O12] Detailed analysis of bacterial and fungal communities of sperm isolated from four different goldfish lines during chilled storage

Nagy, B.[1], Bokor, Z.[1], Kaszab, E.[2], Suhajda, Á.[2], Farkas, M.[3], Bartucz, T.[1], Csorbai, B.[1], Urbányi, B.[1], and Bernáth, G.[1*]

[1]Department of Aquaculture, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary
[2]Department of Environmental Safety, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary
[3]Department of Molecular Ecology, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

*Presenting author: bernath.gergely@uni-mate.hu

Goldfish has a great interest as ornamental fish and as model species. Recent studies highlighted that notable differences can be detected in gamete quality of both sexes between the different commercial lines. However, only limited information is available on the bacterial and fungal communities which can affect the gamete quality, especially during chilled storage. Therefore, three sperm samples were obtained and stored for 96 hours (at 4 °C) from common, shubunkin, black moor and oranda goldfish lines to determine cell counts and to isolate representative bacterial and fungal strains using selective and non-selective media. Microbial communities were identified based on 16S rDNA (bacteria) and 18S rDNA, ITS (fungi) sequencing. The average CFU/mL numbers of sperm samples were similar in all lines both at 0 and 96 hour. At 0 hour, *Aeromonas, Microbacterium, Chryseobacterium* and *Stenotrophomonas* sp. were dominant in all lines. *Brevundimonas, Sphingomonas* sp. were isolated only from common, *Acinetobacter, Micrococcus*, *Glutamicibacter*, *Rhodococcus* sp. from shubunkin, *Serratia* sp. from black moor, and *Exiguobacterium, Comamonas* sp. from oranda goldfish. At 96 hour, *Pseudomonas* and *Aeromonas* sp. were dominant in all lines. *Shewanella* sp. was identified only in common, *Morganella, Haemophilus* sp. in shubunkin, *Serratia sp.* in black moor lines. The *Sphingomonas, Serratia, Micrococcus, Glutamicibacter, Chryseobacterium, Citrobacter, Acinetobacter, Sphingobacterium* sp. showed phenotypic resistance to streptomycin. Regarding the cultivable fungal community, *Penicillium* sp. was isolated from all goldfish lines at 0 hour. *Cladosporium* sp. was identified in oranda samples and *Aspergillus* sp. in common and shubunkin goldfish. After 96 hours, only *Penicillium* sp. was isolated in all lines. According to our results, a diverse and line-specific bacterial, and a slightly uniform fungal community was cultured in the four goldfish lines, which further strengthen the need for microbiological studies in reproductive biology.

Funding: This work was supported by the Research Excellence Programme and the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences. Our work was also funded by the project 2022-1.2.6-TÉT-IPARI-TR-2022-00002 within the framework of the National Research, Development and Innovation Fund announced by the Ministry of Culture and Innovation. The experiments were also supported by the ÚNKP-22-3 New National Excellence Program of the Ministry of Culture and Innovation from the source of the National Research, Development and Innovation Fund.

[O13] Natural extracts from grape marc as supplements for fish semen extenders

Kholodnyy, V.[1], Dzyuba, B.[1], Sotnikov, A.[1], Martínez-Pastor, F.[2], Faldyna, M.[3], Matiašková, K.,[3] and Boryshpolets, S.[1*]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Czech Republic
[2]INDEGSAL, University of León, León, Spain
[3]Veterinary Research Institute, Brno, Czech Republic

*Presenting author: sboryshpolets@frov.jcu.cz

The widespread use of antibiotics has led to the emergence and dissemination of antibioticresistant bacteria worldwide, along with the diminishing efficacy of conventional antimicrobial treatments. The breeding industry, including aquaculture, commonly uses semen extenders containing a variety of antibiotics, which also may contribute to global antimicrobial resistance. Thus, there is a pressing need for farmers to minimize antibiotic usage in livestock production. To address these challenges and support livestock farmers in maintaining healthier animals, the EU Horizon2020-funded project NeoGiANT is developing an innovative solution, harnessing grape marc extracts' antimicrobial and antioxidant properties. In the frames of this project, we tested several extracts, which differed in extraction procedure and final base solvent in terms of the effects on fish spermatozoa during short-term storage. In preliminary experiments, we selected: (1) proper storage media - artificial seminal fluid (ASF) for each species; (2) two grape marc extracts (L and S) and (3) a working concentration of 0.05% (approximately 3 μg/L final polyphenol concentration), which did not interfere with sperm motility directly while still potentially preserving antimicrobial and antioxidant activity during storage. Sperm from 5 to 8 individual common carp, sterlet and rainbow trout males were collected and diluted under the ratio 1:1 with ASF. To estimate the necessity of antibiotic treatment and check the effectiveness of ASF and extract additives, we used the following five storage conditions: 1) non-diluted sperm and diluted in 2) ASF, 3) ASF+antibiotic (Penicillin-Streptomycin; 1 μl/ml), 4) ASF+L, 5) ASF+S. All the samples were stored at 4°C, and the motility of samples was recorded on the 1st, 3rd, and 6th days of storage. On the last day of storage, samples were frozen for further bacteriological analyses, and *in vitro* fertilization trials were performed. Despite the large variability between males, we didn't observe a significant difference in motility and fertilizing ability between storage conditions (except for non-diluted sperm) in all three studied species. Our results suggest that during short-term storage (up to one week, focused on further artificial insemination), the bacterial contamination of the sample does not suppress the spermatozoa motility/viability after storage. Moreover, bacteriological analyses of total bacterial content in samples after the storage suggested the presence of certain antibacterial activity in samples containing extracts compared to pure ASF. In conclusion, our results open the possibility of replacing or reducing antibiotic usage in fish semen extenders in aquaculture.

Funding: NeoGiANT 101036768 (H2020-LC-GD-2020-4)

[O14] Advancing aquaculture and conservation: practical applications of sperm preservation technologies in fish reproduction

Nusbaumer, D.[1*], Karlova, Y.[1], and Karlov, A.[1]
[1]AKYmed Sàrl, Cheseaux-sur-Lausanne, Switzerland

*Presenting author: david@akymed.com

Innovative sperm preservation techniques are central for advancing aquaculture practices and conservation efforts. This presentation details the applications of products and services of AKYmed dedicated to the assessment, and short- and long-term preservation of fish semen. First, we present FISH STOP, our novel short-term sperm preservation medium, highlighting preservation durations and logistical advantages. We explore its efficacy in maintaining sperm viability for weeks in various species and its current application in Eurasian perch aquaculture where it improved production organization, animal welfare and reduced egg loss due to spontaneous female spawning. We also discuss our cryopreservation methods and their efficacies for various species and how it is used both in aquaculture and for biodiversity conservation. We review 3 cases: the strategic semen banking of breeders in fish farming, the use of sperm cryopreservation for preservation and restocking of the endangered European grayling, and the application of this technology for the reintroduction of the extinct Atlantic salmon in Switzerland waters. Furthermore, we introduce QualiSperm, our computer-assisted sperm analysis (CASA) system, illustrating its role in animal breeding and research. The presentation underscores the utility of established technologies and their combined pragmatic application to fulfill contemporary demands in aquaculture and conservation without the complexity of novel scientific development.

Funding: AKYmed

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

WEDNESDAY 17.07.24

Plenary session

[K2] Threats of endocrine disrupting chemicals on fish gametogenesis: mechanisms and biological implications

Carnevali, O.
Department of Life and Environmental Sciences, Università Politecnica delle Marche (UNIVPM), Ancona, Italy

Presenting author: o.carnevali@staff.univpm.it

Endocrine-disrupting chemicals (EDCs) are a group of exogenous compounds that interfere with the hormonal regulation of biological processes. Their pervasive presence in aquatic environments, primarily due to anthropogenic activities, has raised significant concerns regarding their impact on aquatic life, particularly fish. This keynote lecture synthesizes current research on the detrimental effects of EDCs, such as bisphenol A, phthalates, pesticides, and others, on both the production and quality of fish oocytes and spermatozoa. In females, EDCs can lead to altered vitellogenesis, abnormal oocyte development, and increased apoptosis. In males, they can cause disruption of testis architecture, a decrease in sperm count, a reduced sperm motility, and altered sperm morphology. Notably, exposure to EDCs has been linked to alterations in gametogenesis not only in the exposed specimens but also in subsequent generations, as epigenetic changes in the germ cells are inherited by F2 and F3 embryos. These disruptions can compromise fertilization success and subsequent embryonic development, posing a threat to both fish populations and ecosystem health. In this regard, this communication will also highlight examples of long-term and transgenerational effects of EDC exposure in model organisms, along with potential biomarkers for the early detection of endocrine disruptors in aquatic environments.

Our "fish-gametes family" represents the ideal breeding ground for fostering interest and commitment, which are crucial for better understanding the mechanisms of EDC action on fish gametogenesis and for developing effective mitigation strategies to safeguard fish fertility.

Session ENV [Chair: Dr. Oliana Carnevali and Dr. Rafael Nóbrega]

[O15] Unraveling glyphosate toxicity on brain-gonadal axis in zebrafish female: a multiomics study

Giommi, C.[1,2*], Lombó M.[1,2,3], Pinto, G.[4], Serpico, S.[4], Illiano, A.[4], Habibi Hamid, R.[5], Maradonna, F.[1,2], Amoresano, A.[2,4], and Carnevali, O.[1,2]

[1]Dipartimento Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy
[2]INBB—Consorzio Interuniversitario di Biosistemi e Biostrutture, Roma, Italy
[3]Department of Molecular Biology, Universidad de León, León, Spain
[4] Dipartimento di Scienze Chimiche dell'Università di Napoli Federico II, Napoli, Italy
[5]Department of Biological Sciences, University of Calgary, Calgary, Canada

*Presenting author: c.giommi@staff.unipm.it | *Student*

Glyphosate (GLY), the active compound of several herbicide formulations, is commonly used for weed control in crops. Although it was predicted to possess no action against organisms other than plants and bacteria, some evidence demonstrated its detrimental effects on different animal. Since its application widely increased over the years, its accumulation in the environment represents a concrete and severe risk for both wildlife and human health. In order to evaluate the female reproductive toxicity of GLY, an experiment was carried out exposing zebrafish adults through the diet to 0.5 mg/kg body weight (bw)/day (GLY 0.5) defined by the EFSA as acceptable daily intake (ADI), 5 mg/Kg bw/day (GLY 5) and 50 mg/Kg bw/day (GLY 50) displaying no observable adverse effect (NOAEL), for three weeks. The analysis of plasma sex hormones revealed that GLY 50 was able to increase plasma progestins, estradiol (E2) and testosterone (T). Moreover, the exposure to GLY 5 significantly decreased the E2/T ratio. These results highlighted the endocrine disruptive capacity of these doses. Moving to gonadal level, despite the analysis of follicles frequency at different maturation stages was not affected by GLY exposure, the transcript levels of genes involved in oogenesis was instead impacted. The analysis of class IIIb follicles revealed that GLY 50 led to an increase of the gonadotropin receptors (*fshr* and *lhcgr*) and estrogen receptors (*esr1* and *esr2a*) transcripts, suggesting the estrogenic effect of this xenobiotic. In addition, *ccnb1*, localized in the animal pole, and *bmp15*, involved in follicle maturation, were decreased by GLY 0.5 in class IIIb follicles and by GLY 50 in class IV follicles, suggesting a negative impact on oocyte maturation as confirmed by the decreased fecundity found in GLY 50. As for gonadal proteomic analysis, preliminary results indicated that out of 62 proteins identified, GLY was able to downregulate the levels of 23% (GLY 0.5), 32% (GLY 5) and 29% (GLY 50) and to upregulate the levels of 5% (GLY 0.5) and 2% (GLY 50) of proteins compared to Control. At brain level, preliminary proteomic results evidenced that out of 702 proteins identified, GLY downregulated the levels of 8% (GLY 0.5 and 5) and 22% (GLY 50) and upregulated the levels of 60% (GLY 0.5), 55% (GLY 5) and 40% (GLY 50) of proteins compared to Control. Additional bioinformatic analysis are in progress to identify the key molecules responsible of oogenesis process trough brain-gonadal axis. The results of transcriptomic and hormone levels evidenced the endocrine disruptive capacity of GLY, mainly the GLY 50 dose.

Funding: The project received funding from Fondo Ateneo 2022 to OC and FM.

[O16] From brain to sperm: effect of psychoactive drugs on male fish reproduction

Fedorova, G.*, Galicova P., Kholodnyy V., Sotnikov A., and Boryshpolets, S.
University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Water, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, CZ-389 25 Vodňany, Czech Republic

*Presenting author: gfedorova@frov.jcu.cz

There is a growing concern about the occurrence of psychoactive drugs in the aquatic environment, which are introduced to the water by anthropogenic inputs, being only partially metabolized by the human body. They are widely prescribed with a 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. Several studies confirm behavioural changes in fish and crayfish exposed to psychoactive drugs. 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 on fish, they will exhibit similar biological effects across species 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 in both humans and fish. Individual targets are well conserved, suggesting that psychoactive drugs in fish act through similar mechanisms as in humans.

Neuronal signalling is important not only for brain function but also for sperm function. It is regulated by signals, several of which correspond to neurotransmitters that activate the transduction signalling implicated in the molecular control of sperm physiology. Mammalian spermatozoa express receptors for many neurotransmitters and neuromodulators. Information on the presence of neurotransmitters and their receptors in fish sperm and gonads is limited. If NTs may be involved in sperm physiology, then 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. The specific scope of the study was to do a comprehensive analysis of the common neurotransmitters (monoamines, trace amines, amino acids, and others, as well as some of their precursors and metabolites) in the brain, gonads and sperm of the European perch (*Perca fluviatilis*), and then check if they will be altered by the exposure to psychoactive drug methamphetamine. Mature male perch were exposed in aquaria to the environmentally relevant concentration of methamphetamine for 23 days during the spawning season. We observed the bioaccumulation of methamphetamine in all analyzed tissues. Significant changes in some neurotransmitters in sperm were observed when comparing exposed vs control fish. Sperm motility was assessed to check the effect of methamphetamine on sperm function, with no significant difference between control and exposure.

Funding: Czech Science Foundation [project No. 22-03754S], South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses CENAKVA.

[O17] Impact of bisphenols on zebrafish spermatogenesis, sperm quality, reproduction and embryo development

Costa, D. F.[1], Borali, L. M.[1], Camargo-dos-Santos, B.[1], Bellot, M. S.[1], Giaquinto, P. C.[1], Habibi, H. R.[2]*, and Nóbrega, R. H.[1]
[1]Department of Structural and Functional Biology, São Paulo State University, Botucatu, Brazil
[2]Department of Biological Sciences, University of Calgary, Canada

*Presenting author: habibi@ucalgary.ca

Abstract: Bisphenol A (BPA) is a widely used plasticizer that has been extensively studied due to its adverse action on the hypothalamic-pituitary-gonadal axis. Alternative bisphenols, such as bisphenol-S (BPS) and bisphenol-AF (BPAF), have been developed to replace BPA. Recent studies have shown that these compounds may also act as endocrine disruptors. In the present study, adult male zebrafish were exposed to BPA alternatives, BPS and BPAF for 14 days. We investigated the effects of exposure to these bisphenols on 11-KT production, spermatogenesis, and sperm quality. We subsequently crossed the exposed male fish with untreated females and evaluated the offspring's hatching, development, survival, and gene expression. Similar parameters were also investigated in embryos directly exposed to BPS or BPAF. We also performed in vitro exposure using ex vivo cultured testis with increasing concentrations of BPS and BPAF for seven days. We then evaluated spermatogenesis using a histomorphometric approach and assessed the expression of several genes involved in gonadal function. Our results demonstrate the disruption of androgen-mediated testicular function in zebrafish exposed to BPS and BPAF. Both in vitro and in vivo BPS and BPAF treatments altered the expression of steroidogenic enzymes, including *cyp19a1a, star,* and *cyp7a1*, and modulated the expression of epigenetic regulatory enzymes. In vivo exposure increased meiotic (spermatocytes) and post-meiotic (spermatids) cysts, leading to an abnormal increase in spermatozoa production. We also observed adverse actions of the contaminants on sperm quality, motility, and embryo viability. In the F1 generation, there was a marked delay in hatching rates, increased mortality, and malformation. This study provides evidence supporting the hypothesis that BPS and BPAF exert epigenetic effects and adversely impact reproduction by altering steroidogenesis and spermatogenesis. In vitro, exposure to BPS and BPAF reduced the frequency of primary spermatocytes in pachytene cysts and altered the expression of estrogen-related genes, including *esr1, cyp19a1b,* and *vgt1*, indicating direct action of the contaminants at the level of the testis. Overall, our study provides information on the mechanisms underlying the harmful effects of bisphenol alternatives on reproduction in fish.

Funding: Funded by NSERC Grants to HR Habibi and São Paulo Research Foundation grant to RH Nobrega.

[O18] "Fish Wars" in xenoestrogenic waters: "The Ribosome Awakens" in the oocyte

Bir, J., Urrutia A., Diaz de Cerio, O., Ortiz-Zarragoitia, M., and Cancio, I.*
CBET+ Research Group, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country. Areatza Hiribidea 47, 48620 Plentzia

*Presenting author: ibon.cancio@ehu.eus

Chelon labrosus mullets from polluted transitional waters in South Biscay Bay show high prevalence of intersex condition, producing previtellogenic perinucleolar oocytes (Oo) in testes. A morphological feature of such Oo is the amplification of nucleoli, indicative of rRNA production and ribosomal subunit assembly. Oo are unique cells in metazoans as they accumulate molecules to be used by another individual, the embryo. Protein synthesis is one of the energetically most demanding processes in a cell and Oo do part of the job for the embryo by contributing the necessary ribosomes. RNA electrophoretic profiling in ovaries (Ov), testes (Te) and intersex testes (IT) of mullets from Gernika estuary shows that RNA polymerase-III products, 5S rRNA and tRNAs, are potent markers of the presence of Oo. The analysis of such non-coding RNAs (ncRNAs) relative to the presence of 45S rRNA maturationproducts serves to rank IT gonads according to the intersex-severity. Fish genomes possess multiple tRNA gene-copies (>15000 in *D. rerio*) coding for the 61 codon-specific aminoacyltRNAs. The complement of tRNAs transcribed (isoaceptors and isodecoders) in the Oo could modulate the population of mRNAs that are going to be translated in the embryo. Sequencing of this "tRNA-ome" is technically complicated, due to the structural characteristics, level of post-transcriptional modifications and variability of tRNAs. Thus, we characterized the ncRNAs in Ov, Te and IT of mullets via a miRNA-Seq approach. A total 247 miRNAs were identified, expression levels of 47 distinguishing Ov from Te. While only 14 miRNAs differed in IT vs Ov, 47 varied (17 up, 30 down) in IT vs Te, some related to ribogenesis. The approach also provided reads of other small ncRNAs, most of them involved in processes taking place in the nucleolus. Ov and IT showed higher levels of 5S rRNAs, tRNAs and snoRNAs than Te. 95 different snoRNAs, and all major snRNAs (U1, U2, U4, U5 and U6 spliceosomal RNAs), were sequenced. Their expression profile clustered Ov and IT apart from Te. The only snRNA transcribed by Pol-III, U6, was enriched in Te. Among tRNAs, total read profile showed high variability across individuals. Only isoaceptors with different levels of transcription between groups were Leu_TAG, Lys_TTT and Suppress_TCA. Thus, the high levels of ncRNAs pinpoint their upregulation due to mullet feminization under xenoestrogenexposure and flag ribogenesis-control as a crucial process during Oo differentiation. Molecular tools based on these ncRNAs are proposed as markers of feminization in xenoestrogens exposed fish, and also for the analysis of oogenesis and Oo quality.

Funding: Basque Gov. (IT1743-22), MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

[O19] They're here: current heat waves arrest rainbow trout (*Oncorhynchus mykiss*) spermatogenesis

Calvo-Rodríguez, L.[1*], Ortiz-Delgado, J. B.[2], de Paz, P.[1], Fernández, I.[3], and Riesco, M. F.[1]

[1]Cell Biology Area, Department of Molecular Biology, Universidad de León (ULE), León, Spain
[2]Instituto de Ciencias Marinas de Andalucía-ICMAN/CSIC, 11510 Puerto Real, Spain
[3]Centro Oceanográfico de Vigo (IEO-CSIC), 36390 Vigo, Spain

*Presenting author: lcalr@unileon.es | *Student*

Climate change poses serious short and medium-term threats to aquatic environments. In fish, as ectothermic organisms, many biological processes can be affected by temperature. Among them, gametogenesis is a highly temperature-sensitive process. The present study is focused on elucidating the potential effects of heat wave phenomena, a more realistic approach of climate change, particularly considering fish spermatogenesis. For this purpose, two experimental groups were established: i) trout males subjected to a heat wave event (H-W): with a progressive water temperature increase (1 °C day-1), until reaching 20 °C, maintained for eight days and finally the initial temperature (14 °C) being restored; and ii) Control fish (Control), constantly maintained at 14 °C. Samples from rainbow trout Control and H-W male breeders were taken at two sampling points: sampling 1 (S1), climax of the heat wave; and sampling 2 (S2), when normal temperature was restored. The potential effects of a single heatwave event have been evaluated by means of biometric, redox and stress response, blood plasma testosterone, sperm motility, histopathology and gene expression analyses in both groups.

In the H-W group decreased body weight and total antioxidant status, but increased cortisol levels and redox enzymatic activities in blood plasma were found compared to Control males. The experimentally recreated heat wave also decreased testosterone plasma levels and sperm motility, arrested spermatocyte type I and II differentiation and increased spermatozoa apoptosis in H-W exposed males. Consequently, we decided to investigate the molecular alterations that trigger this spermatogenesis failure in H-W males. RNA-Seq analysis of testes showed as in 116 and 1040 genes were up- and down-regulated in H-W males (fold change ≥ 1.5; p adjust value < 0.01). GO analysis showed that apoptosis KEGG pathway was positively overrepresented, while sperm axoneme assembly and other biological processes related to sperm functionality were negatively overrepresented. Present results suggest that heat wave events threaten reproductive performance and therefore might compromise aquaculture growth and sustainability.

Funding: Work funded by REPHEAT (PID2021-127782OA-I00) project MCIN/AEI/10.13039/501100011033, FEDER "Una manera de hacer Europa" and TED2021-131893B-I00, AEI/10.13039/501100011033/ Unión Europea NextGenerationEU/PRTR.

[O20] Effective challenge test to select resilient males of Mediterranean aquaculture fishes to environmental changes

França, T. S.[1*], González-López, W. A.[1], Fernández-García, F.[1], Belenguer, A.[2], Holhorea, P. G.[2], Calduch-Giner, J. C.[2], Felip, A.[2], Gómez, A.[2], Mañanós, E. L.[2], Pérez-Sánchez, J.[2], Pérez, L.[1], and Asturiano, J. F.[1]

[1]Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain

[2]Instituto de Acuicultura Torre de la Sal (IATS, CSIC), Ribera de Cabanes, Castellón, Spain

*Presenting author: thalesfranca@gmail.com

In the context of global warming, we aimed to evaluate the effect of temperature and pH of artificial seawater (ASW) on the activation of sperm from different aquaculture Mediterranean species: gilthead seabream *Sparus aurata*, European sea bass *Dicentrarchus labrax*, and sole *Solea senegalensis*. Furthermore, that sperm evaluation was used to select resilient males, and to form a germplasm bank composed of resistant samples to environmental changes. Sperm samples were collected and diluted in an extender at 1:25 (sperm:diluent). The sperm samples that showed >50% motility (MOT, %) were selected using a CASA-Mot system. The samples were subjected to three challenge tests. The first one was the sperm activation using ASW at different pH (6.5, 7.2, 7.4, 7.6, 7.8, 8, 8.2, 9.5). The second challenge test was activation with ASW at different temperatures (seabream and sole: 4, 16, and 22 °C; sea bass: 4, 12, and 20 °C). The third one evaluated the combined effect of ASW pH and temperature; pH (7.8 and 8.2) x (seabream and sole: 4, 16, and 22 °C; sea bass: 4, 12, and 20 °C). The third challenge test evidenced the presence of sperm samples able to resist these environmental changes, and allowed the selection of males being resilient to these climate change effects. The sperm MOT values obtained under natural environmental conditions [(pH 8.2; temperature: 16 °C (seabream and sole) and 12 °C (sea bass)] were used as a reference. Then, to evaluate the individual sperm samples, different environmental parameters were applied, and the percentage of variation of MOT values in comparison with the reference values were determined. Then, more or less strict criteria can be used, considering different percentages of MOT variation (i.e.: 5 or 10%) for the selection of resilient males. The decrease in ASW pH impaired the MOT of sea bream sperm, and the temperature increase affected the sea bass and sole sperm. Acidification and warming of the marine environment affects the sperm quality of the three Mediterranean species. The assessment of sperm samples with challenge tests proved to be an effective tool for

[O21] Impacts of global warming on fertility of male fishes: evidence from meta-analysis of temperature effects on spermatozoa motility

Cheng, Y.[1*], Wang, J.[2], Alavi, S. M. H.[1,3], Zhang, S.[1], Linhartová, Z.[1], Roy, D. R.[1], Shazada, N. E.[1], Dzyuba, B.[1], Linhart, O.[1]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, Vodňany 389 25, Czech Republic
[2]School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, 203 Swingle Hall, Auburn, AL 36849, USA
[3]School of Biology, College of Science, University of Tehran, Enqhelab Avenue, Tehran 14176-14411, Iran

*Presenting author: ycheng@jcu.cz

Studies have demonstrated adverse effects of global warming on aquatic ecosystems from the abiotic to the biotic level. Meta-analysis is widely used in clinical research, although it is less prevalent in basic biology. However, it is increasingly demonstrating its utility in the context of certain basic research and is expected to enhance the reproducibility of research findings. In the present work, a meta-analysis study was conducted to elucidate the effects of global warming on spermatozoa functions, which are key determinants of male fertility. We recruited 245 data records from a pool of empirical studies, which includes 20 studies spanning 20 cold- and warm-water fish species, to identify the effects of increased water temperature (IWT) on determinants of sperm fertility in fishes. The data were systematically re-processed and re-analyzed to determine the overall effects of IWT on sperm kinetics such as motility (MOT), duration of motility (DSM), curvilinear velocity (VCL), rectilinear velocity (VSL) and average path velocity (VAP), as well as on enzymatic activities for energy supply (EAES) and antioxidant enzyme activity (ANEA). The standardized mean difference was calculated for each study, with positive values indicating higher performance under IWT. The results showed that (a) the overall effect size for MOT was more negative in cold-water fishes (-1.22) than in warm-water fishes (-0.98). (b) Each 1 $_{o}$C increase in the activation medium reduced MOT by 1.30% (cold-water fishes) and 3.47% (warm-water fishes). (c) The IWT negatively affected DSM, decreasing it by 10 s (cold-water species) and 5.64 s (warm-water species) per degree of IWT. (d) Spermatozoa velocity (VCL and VSL) was increased by IWT in warm-water species. In conclusion, this study shows that IWT negatively affects sperm motility kinetics, suggesting an impact of global warming on fish reproduction.

Funding: Ministry of Education, Youth and Sport of the Czech Republic (LM2023038), Czech Science Foundation (23-06426S), National Agency for Agriculture Research (QK21010141).

Session QUAL [Chair: Dr. Catherine Labbé and Dr. Daniel Żarski]

[O22] The presence of inclusions in blastodiscs of coho salmon embryos (*Oncorhynchus kisutch*) is associated with low rates of fertility and embryo survival

Sandoval-Vargas, L.[a], Pérez-Atehortúa, M.[a], Figueroa Villalobos, E.[b], Schulz Fontealba, F.[c], Schulz Ferrada, M.[a,d], and Valdebenito, I.[a*]

[a]Faculty of Natural Resources, Catholic University of Temuco, Temuco 4813302, Chile
[b]Nucleus of Research in Food Production, Faculty of Natural Resources, Catholic University of Temuco, Temuco, 4813302, Chile
[c]Instituto Alemán Carlos Anwandter. Valdivia. Chile
[d]Marine Farm Tornagaleones S.A. Pto. Montt. Chile

*Presenting author: ivisler@uct.cl

In species with long incubation periods, such as salmonids, it is necessary to perform early evaluation of the egg quality to optimize space and investment. In Chile, some salmonid breeding companies habitually evaluate both the early fertilization rate and the cleavage pattern. Characteristics of the first blastomeres in fish have been evaluated in previous studies as a tool for predicting egg and larva quality. Nevertheless, there are still few studies of the presence of inclusions in fish blastodiscs or blastomeres. The object of the present study was to describe the relationship between cellular inclusions and some embryo quality parameters (symmetry, fertility rate, survival rate) in farmed coho salmon. Eggs from 261 females were fertilized with 400 × 10$_3$ spermatozoa/egg and incubated in the dark in separated flow through incubators. Embryos at the fourcell stage were fixed in Stockard's solution and the blastodiscs were classified as: i) unfertilized, ii) fertilized with inclusion, and iii) without inclusion. The blastomere symmetry was also recorded. Embryos from 30 females (15 with and 15 without inclusions) were incubated until pigmented eye embryo stage to determine the survival rate. Cell inclusions were detected in 8.81% of the females evaluated, with 24.1± 24.2 of the eggs from each of those females presenting inclusions. These structures are always found in the intracellular space and can be observed as one or several holes present in both blastomeres and blastodiscs. The inclusions are variable in size and depth; they can occupy up to about 50% of the blastodisc volume, or can pass right through the cell. The fertility rate in egg samples with inclusions was significantly lower (53.7 ± 22.7%) than that obtained in eggs without inclusions (92.1 ± 7.9%). Similarly, the blastomere symmetry and the survival rate were significantly lower when the blastomeres contained inclusions (38.7 ± 19.4% and 52.2 ± 33.9 respectively) compared to those without inclusions (58 ± 156 13.3% and 91.2 ± 6.8% respectively). There was also a significant negative correlation between inclusions and fertility, suggesting that as the percentage of inclusions increases, the fertility percentage decreases. In the current study, the presence of inclusions was associated with embryo malformations and low survival. A further area for future studies would be assessment of oocytes in different age stages, to verify the incidence of these structures.

Funding: FONDECYT REGULAR 1211246 and FONDECYT INICIACIÓN 11230690.

[O23] Transgenerational inheritance of DNA methylation alterations after gdf6b-/- -induced sex reversal in the medaka (*Oryzias latipes*)

Depincé, A.[1], Murat, F.[1], Thermes, V.[1], Brionne, A.[1], Schartl, M.[2], and Herpin, A.[1]
[1]INRAE, UR1037 Fish Physiology and Genomics, Rennes F-35000, France
[2]Department of Physiological Chemistry, University of Würzburg, D-97074 Würzburg, Germany

*Presenting author: alexandra.depince@inrae.fr

Fish exhibit a wide range of sex determination mechanisms, coupled with a high turnover of master sex-determining genes. As a result, the downstream molecular pathways and regulations governing sex determination and differentiation have undergone iterative adaptations over the course of evolution. Interestingly, various members of the TGF-ß family (including Amh, Gsdf, Gdf6, Bmpr1bb, Amhr2…) have played pivotal roles in the emergence of most sex-determining genes in fish.

Focusing on the functions of Gdf6 to potentially unveil new conserved mechanisms regulating sex determination, we discovered that the medaka GDF6 paralogs control gonadal commitment in an (unforeseen) heritable epigenetic manner.

Hence, while GDF6 CRISPR/Cas9-mediated knockout fish undergo XY male-to-female sex reversal, we demonstrated that this sex reversal is indeed correlated with profound rearrangements in the entire epigenetic landscape, including the hypermethylation of parts of the Y chromosome, leading to the inactivation of the master sex-determining gene (Dmrt1bY) in medaka.

Further investigations established that XY male-to-female sex reversal occurs only when:

i) the gamete carrying the mutation also carries the Y chromosome, or when

ii) independently of the GDF6 mutation, the Y chromosome is inherited from an already sex-reversed XY female.

Most importantly, this phenomenon of transgenerational sex reversal is abolished when embryos are either treated with the DNA demethylating compound 5-azacytidine or subjected to targeted CRISPR-dCas9-TET1 epigenetic editing. This suggests that the sex-reversal process might be driven and transmitted through epigenetic alterations. In accordance with these findings, overexpression of GDF6b in a medaka cell line results in the nuclear-to-cytoplasmic delocalization of DNMT1 and UHRF1 proteins, two key factors known to be involved in the maintenance of the whole DNA methylation landscape.

Finally, transcriptomic analyses suggest that GDF6-induced epigenetic imprinting could be mediated in an original way by a phylogenetically conserved family of transcription factors.

Taken together, our data strongly support the idea that GDF6 signalling, uniquely transmitted through gametes in a transgenerational and persistent manner, is physiologically integrated by safeguarding the methylation states of various *loci* within the genome, ultimately influencing sex determination.

[O24] Ovarian fluid affects interspecific hybridization between sterlet and Siberian sturgeon

Kholodnyy, V.*, Sotnikov, A., Dzyuba, V., Dzyuba, B., and Boryshpolets, S.
University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic

*Presenting author: vkholodnyy@frov.jcu.cz

The influence of ovarian fluid (OF) on spermatozoa motility characteristics has been studied in various fish species, revealing its impact on sperm performance. In salmonids, the OF presence enhanced spermatozoon velocity, trajectories, and longevity, while in other fishes, the male gametes showed diverse reactions. Earlier, we found inhibitory effects of OF on spermatozoon motility in acipenserids and supposed its contribution to sperm competition dynamics involving multiple males. Such dynamics may be crucial for controlling hybridization events, which can occur not only during artificial fertilization but also under natural conditions, especially due to environmental changes caused by human activity. In this study, we examined the impact of OF on hybridization between sterlet (*Acipenser ruthenus*) and Siberian sturgeon (*A. baerii*), as well as on spermatozoa motility. *In vitro* fertilization trials were conducted with sterlet eggs and sterlet and Siberian sturgeon spermatozoa in the presence/absence of the OF, and with rival sperm added simultaneously or one after the other after a time delay. The results revealed that in the presence of sterlet OF, sterlet sperm exhibited higher motility and velocity compared to Siberian sturgeon sperm. This selective advantage resulted in a various proportion of hybrid/non-hybrid embryos depending on the experimental conditions and the presence of sperm competition. Specifically, the proportion of hybrid embryos decreased significantly in the presence of ovarian fluid, supporting the hypothesis that OF promotes conspecific sperm and may act as a barrier to hybridization. Conversely, when OF was absent, the hybrid embryo proportion increased, indicating less selective fertilization. The study confirmed that the fertilization rate was influenced by the timing of sperm-egg contact, and the hybridization rate depended on rival priority. Nevertheless, the advantage of conspecific spermatozoa was obvious in all tested conditions. Chemotaxis experiments showed no significant attraction of either sperm type to the non-diluted sterlet OF, suggesting that the selective mechanism was likely not chemotactic but due to differences in motility activation and maintenance in the presence of ovarian fluid. The findings suggest that OF in sturgeons may act as a selective medium favoring conspecific sperm and potentially serving as a natural barrier to hybridization.

Funding: Ministry of Education, Youth and Sports of the Czech Republic (project "CENAKVA ", LM2018099).

[O25] Maternal contributions to offspring immunity: beyond direct transfer

Rocha de Almeida, T.[1*], Schulz, P.[2], Pajdak-Czaus, J.[3], Nynca, J.[1], Grecka, K.[4], and Żarski, D.[1]

[1]Polish Academy of Sciences - IARFR, Olsztyn, Poland
[2]National Inland Fisheries Research Institute, Olsztyn, Poland
[3]University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
[4]Dabie Fish Hatchery, Bytow, Poland

*Presenting author: t.almeida@pan.olsztyn.pl

Maternal effect happens when females' phenotype helps to shape offspring phenotype beyond genetically transmitted features. An important maternal effect is the maternal-offspring immunity transmission or [the maternal side of] transgenerational immune priming (TGIP). Maternal transfer of immunoglobulins (Ig) is the most known and studied TGIP mechanism. However, other mechanisms shaping immune response in fish remains to be unveiled. Here, we tested the potential influence of egg content on offspring's ability to mount a proper immune defense against *Yersinia ruckeri*. Two strains of rainbow trout (*Onchorynchus mykiss*) were studied from egg fertilization to 6g juveniles when the bacterial challenge was performed. Unfertilized eggs were snap frozen after collection for transcriptomic analysis and several parameters of early and late offspring performance were recorded. Additionally, gills, liver and spleen were collected during challenge for gene expression analysis. High quality eggs were obtained from both strains and no differences between them was observed until hatching. In growth performance, however, difference was observed between strains from the fifth week. At the end of growth period, strain A and B presented, respectively: 6.0±1.2g and 9.4±2.0g. No mortality difference during this period was observed between strains. During bacterial challenge, mortality in strain B started earlier and was three times higher (66.7±0%) than the one observed in strain A (13.6±0.6%). No mortality was observed in the control group for both strains. Eggs transcriptomes revealed 537 differentially expressed genes (DEG) between strains. Of those, more than 30% have immune related gene ontology annotations. Gene expression analysis in the organs collected during challenge was thus focused on selected immune related DEG in eggs. Expression differences between control and infected juveniles showed that all genes tested responded to the experimental infection by changing their expression level in at least one of the organs. Noteworthy, three genes (*spi1*, *ctss* and *itgb2)* responded to the infection in all three organs and were also different between strains. Our data suggest that maternal effects extend beyond the classical direct transfer of maternal Ig despite being largely erased during embryonic early development. Therefore, we infer that differences in maternal transcriptomic cargo may have further consequences, potentially shaping physiological status, including the immune system, in a cascade-like manner.

Funding: Horizon 2020 under Marie Skłodowska-Curie grant (No 847639) and Ministry of Education and Science. Dabie Hatchery provided fish strains.

[O26] Parental dispute over progeny phenotype in Eurasian perch

Panda, A.[1*], Debernardis, R.[1], Palińska-Żarska, K.[2], Judycka, S.[1], Klopp, C.[4], Almeida, T. R. D.[1], Jarmołowicz, S.[2], Hliwa, P.[3], and Żarski, D.[1]
[1]Institute of Animal Reproduction and Food Research, Olsztyn, PAN, Poland
[2]National Inland Fisheries Research Institute, Oczapowskiego 10, 10-719 Olsztyn, Poland
[3]University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland
[4]Sigenae, UR875, INRAE, Castanet-Tolosan, France

*Lead presenter: a.panda@pan.olsztyn.pl | *Student*

Parental contribution on progeny is influenced by a complex web of relationships between genetic and non-genetic (regulated by the environment) inheritance factors. In this context, parental experiences (e.g., food quality and availability) can lead to phenotypic consequences in their progeny (e.g., size of progeny). Domestication (DOM), being a dynamic process of physiological and behavioral adaptation to man-controlled environment, impacts direct phenotypic consequences stemming from, among others, human intervention into the life cycle of fish. Consequently, progeny of DOM individuals constitutes considerably distant phenotype from wild ones exhibited notably in survival and growth rates during rearing in human-controlled environment. Recent studies show, that DOM-related phenotypic modifications are easily detectable also at the molecular level (gene expression) in larvae at hatching, making it an excellent tool to explore parental control over gene expression in the progeny. The aim of this study was to identify repertoire of transcripts being under either paternal or maternal control while using wild (W) and DOM stocks of Eurasian perch constituting extremely different phenotypes. Crosses namely: DOM females x Wild males (DW), DOM females x DOM males (DD); and Wild females x DOM males (WD), Wild Females x Wild males (WW) were made. After hatching, they were reared in controlled conditions following standardized protocols. For the experiments, cryopreserved semen from wild and domesticated males were used, to use the same set of males every time. For all kinds of analyses, the comparisons were done in the following manner: DD vs WD, DW vs WW (as maternal effect) and WD vs WW, DD vs DW (as paternal effect). Zootechnical parameters such as length, weight, cannibalism, mortality, swim bladder inflation effectiveness, showed significant statistical differences stemming from maternal effect. Transcriptomic data of whole larvae (at mouth opening stage), revealed lower number of differentially expressed genes (DEGs) from paternal side (DD vs DW = 22 DEGs, WD vs WW = 5 DEGs), in comparison to maternal side were considerably higher (DD vs WD = ~800 DEGs, DW vs WW = ~2000 DEGs). Overall, this study though indicates considerable maternal dominance in shaping progeny phenotype. However, interestingly, 20 (out of 27) identified paternal-effect-genes are overlapping with maternal effect ones. This study sheds light on remarkable parental dispute over shaping transcriptomic profile of the progeny where considerable modulatory role of both parents is seen, but requires further exploration.

Funding: This work was funded by National Science Center of Poland (SONATA BIS project, number UMO-2020/38/E/NZ9/00394).

[O27] Exploring new markers of gamete quality: characterization of extracellular vesicles (EVs) in fish seminal and blood plasma

Tsakogiannis, A.[1], Marrero, C.[1], Fatsini, E.[1], Oliveira, C. C. V.[1], Magalhães-Raposo, C.[1], Rbbani, G.[2], Fernandes, J.[2,3], and Cabrita, E.[1*]

[1]Center for Marine Sciences-CCMAR, University of Algarve, Campus Gambelas, Faro, Portugal
[2]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
[3]Institute of Marine Sciences (ICM-CSIC), Spanish Scientific Council, Barcelona, Spain

*Presenting author: ecabrita@ualg.pt

Extracellular vesicles (EVs) are lipid bilayer nanovesicles released by functional cells to body fluids, containing bioactive molecules as cargo. EVs have essential functions in intercellular communication by regulating different biological processes in target cells. Biological fluids such as seminal or blood plasma contain many EVs that are morphologically heterogeneous, and particularly in seminal plasma they can encapsulate proteins and miRNAs that modulate sperm functions and male fertility. In several mammalian body fluids, EVs and their cargo have been identified as putative biomarkers of reproductive performance, however in fish not much information have been published. The present study explores the methodology used to isolate and characterize EVs in fish fluids, focusing on seminal and blood plasma with application on downstream studies. Sperm and blood were collected during reproductive season, centrifuged to obtain plasma, and at least 500 µl were immediately stored at -80ºC. Seminal plasma EVs (SP-EVs) were isolated from turbot (n=10), halibut (n=10) and seabream (n=12); blood plasma EVs (BP-EVs) were isolated from sole (n=20) and turbot (n=10), both fluids using size exclusion chromatography (SEC). EVs were characterized by size distribution (diameter), concentration and zeta potential using tunable resistive pulse sensing (TRPS, Exoid, Izon). For downstream applications, EV-cargo was characterized to identify putative markers. EV-isolates were concentrated with qEV Magnetic Concentration Kit (Izon) and RNAs extracted using qEV RNA extraction Kit (Izon). Libraries were prepared using NEXTFLEX® Small RNA-Seq Kit v4. Normalized libraries were sequenced using the NextSeq500 platform (Illumina). EV-proteins were extracted and tryptic peptides were separated and analysed by LC-MS/MS. Proteins were identified using Fragpipe software at an FDR < 1%. Regarding SP-EV size, the mean values ranged from 141.3 nm (halibut) to 144.16 nm (seabream) and 118 nm (turbot) with $3.9x10_{10}$ EVs/ml, $1.2x10_{10}$ EVs/ml and $0.6x10_{10}$ EVs/ml, respectively. For BP-EVs, mean size was 107.8 nm for sole and 100 nm for turbot. SP-EVs surface are mostly negatively charged with a mean charge of -17.22 mV (seabream) and -15.6 mV (turbot). This means that EVs are in the range of stability since only values bellow -+5mV indicate processes of EV aggregation. Regarding SP-EV proteins, we found 245 specific proteins for turbot and 1139 for halibut, sharing 288 proteins between both species. In terms of miRNAs, 37 and 84 were specifically found in halibut and seabream, respectively. Our results suggest that EVs-cargo characterization could be a useful tool to apply in further experimental approaches such as breeders nutritional studies or hormonal inductions.

Funding: Projects BREEDFLAT (EEA grants; PT-INNOVATION-0080) and SPERMANTIOX (FCT; PTDC/CVT-CVT/4109/2020); UIDP/04326/2020, LA/P/0101/2020.

[O28] Aquaporin-3a dysfunction impairs osmoadaptation in post-activated marine fish spermatozoa

Chauvigné, F.[1,*], Castro-Arnau, J.[1], López-Fortún, N.[1], Sánchez-Chardi, A.[2], Rutzler, M.[3], Calamita, G.[4], Finn, R. N.[1,5], and Cerdà, J.[1]

[1]Institute of Marine Sciences, Spanish National Research Council (CSIC), 08003, Barcelona, Spain, and Institute of Biotechnology and Biomedicine (IBB), Universitat Autonoma de Barcelona, 08193 Bellaterra, Spain
[2]Servei de Microscopia, Universitat Autonoma de Barcelona, 08193, Bellaterra, Spain
[3]ApoGlyx, Lund 22381, Sweden, and Division of Biochemistry and Structural Biology, Department of Chemistry, Lund University, 22100 Lund, Sweden
[4]Department of Biosciences, Biotechnologies, and Environment, University of Bari "Aldo Moro," 70125 Bari, Italy
[5]Department of Biological Sciences, University of Bergen, 5020 Bergen, Norway

*Presenting author: chauvigne@icm.csic.es

Spermatozoon volume regulation is an essential determinant of male fertility competence in mammals and oviparous fishes. In mammals, aquaporin water channels (AQP3 and -7 and -8) have been suggested to play a role in spermatozoon cell volume regulatory responses. In contrast to mammals, the ejaculated spermatozoa of marine teleosts experience a high hypertonic shock in seawater, resulting in an Aqp1aa-mediated water efflux, cell shrinkage and the activation of motility. However, the potential involvement of other aquaporins in cell volume regulatory mechanisms in post-activated marine spermatozoa are unknown. Using the gilthead seabream (*Sparus aurata*) as a model of marine teleosts and a paralog-specific antibody, we show that the AQP3 ortholog Aqp3a is highly accumulated in the mid posterior region of the spermatozoon flagella. Aqp3a-mediated water conductance is specifically inhibited by a recently developed AQP3 antagonist (DFP00173) as well as by the Aqp3aspecific seabream antibody (α-Aqp3a) when expressed in *Xenopus laevis* oocytes. Both DFP00173 and α-Aqp3a impair post-activated sperm motility kinetics (% motility, % progressivity, and curvilinear velocity) with respect to the controls at 30 s post activation in a dose-dependent manner. Interestingly, in a close resemblance to the phenotypes of AQP3-deficient murine sperm, field emission scanning and transmission electron microscopy revealed that both Aqp3a inhibitors induce an abnormal sperm tail morphology, including local swelling and tail angulation, with complete coiling of the flagella in some cases. These data suggest that Aqp3a may function as an osmosensor to regulate cell volume under a high hypertonic stress, or that the channel is required for the efflux of specific solutes which can inhibit sperm motility.

Funding: Supported by the Spanish Ministry of Science, Innovation and Universities (PID2022-138066OB-I00 to J.C., BES-2017-080778 to J.C-A., and CEX2019-000928-S to ICM).

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

THURSDAY 18.07.24

Session GAM [Chair: Dr. Diego Crespo and Dr. Rüdiger Schulz]

[O29] Differential regulation of ribogenesis genes throughout oogenesis in thicklip grey mullets (*Chelon labrosus*), preparing the maternal contribution to embryo development

Bir, J.*, Diaz de Cerio, O., Ortiz-Zarragoitia, M., and Cancio, I.
CBET+ Research Group, Dept. Zoology & Animal Cell Biology; Faculty of Science & Technology and Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country. Areatza Hiribidea 47, 48620 Plentzia, Bizkaia, Spain

*Presenting author: joyanta.bir@ehu.eus | *Student*

Oocytes in teleost fish undergo complex processes of differentiation during oogenesis that involve incorporation of molecules such as ribosomal RNAs that will facilitate the growth of the future embryo. A healthy oocyte must accumulate sufficient amount of ribosomes to allow embryo protein production upon fertilization. Transcription of 5S rRNA and tRNAs is governed by RNA polymerase III (Pol-III), whose activity is modulated by general transcription factors (Gtf3a, b, and c) and one inhibitory protein (Maf1). In previous studies across different teleost species, we have shown that the high expression levels of 5S rRNA and tRNAs in oocytes, easily quantifiable in fish gonads in the way of a 5S rRNA/18S rRNA index, serve as reliable marker to differentiate males and females and also to identify the intersex condition in mullets exposed to xenoestrogens. Hereby, we analysed whether the tRNA/5,8S rRNA index shows the same profile as 5S rRNA/18S rRNA index during oogenesis in the synchronous developing *Chelon labrosus*. We certified that the index values are high during previtellogenesis when only perinucleolar oocytes are present dropping upon secondary growth towards vitellogenesis. Additionally, we analysed the ovarian pattern of transcription of the Gft3b polypeptides coding genes (*brf1a & b, brf2a & b, bdp1 and tbpl2*), in comparison to the previously characterised *gtf3ab*, and also that of the *maf1* gene throughout oogenesis. qPCR analysis revealed that most of the Gtf3b related genes show high transcription levels in previtellogenic ovaries decreasing significantly in cortical alveoli stage ovaries and further more in advanced vitellogenic ones. Therefore, these genes products participating in the regulation of ribogenesis and protein synthesis are efficient molecular tools that can be measured through RNA electrophoresis or qPCR to identify the maturation stages of mullet ovaries. Our results demonstrate that primary growth of fish oocytes is marked by a very strong activation of Pol-III activity.

Funding: Basque Gov. (IT1743-22), MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

[O30] Virginity's role in shaping egg quality and maternal transcript regulation in pikeperch

Nynca, J.[1*], Hliwa, P.[2], Palińska-Żarska, K.[3], Molcan, T.[1], Lewandowski, R.[4], and Żarski, D.[1]

[1]Institute of Animal Reproduction and Food Research, PAS, Olsztyn, Poland
[2]University of Warmia and Mazury in Olsztyn, Poland
[3]National Inland Fisheries Research Institute, Olsztyn, Poland
[4]Fish Farm, Mikołajki, Poland

*Presenting author: j.nynca@pan.olsztyn.pl

The pikeperch is a key predator in open water ecosystems and holds substantial commercial value. Due to the intense pressure from both commercial fisheries and recreational angling, specific regulations have been enacted to protect smaller specimens, ensuring that females spawn at least once in their lifetime before being captured. However, our study on domesticated stocks reveals that the first reproductive event often exhibits reduced quality, raising serious concerns about the effectiveness of current regulations in sustaining pikeperch populations. Therefore, in this study we attempted to investigate reproductive performance (FR-fertilization rate, HR- hatching rate) of identified virgin (three-year-old; n=6) and experienced females (four-year-old; n=6). Besides, we have performed transcriptomic analysis of unfertilized eggs in order to unveil processes responsible for lowered egg quality and to identify molecular markers of virginity in pikeperch, which may become a powerful tool for monitoring both wild and domesticated stocks. Eggs from virgins were characterized by the abnormally small-sized eggs (about 10%) and significantly lower quality ($p<0.05$; FR: 63%, HR: 49%) compared to experienced fish (FR: 88%, HR: 75%). Significant differences were also observed at the transcriptomic level between eggs. A total of 270 differentially expressed genes (DEGs; FDR <0.01) were identified, comprising 17 downregulated and 253 upregulated DEGs in eggs from virgin fish. Gene Ontology analysis of DEGs revealed translation and energy generation as the most enriched biological process. The upregulation of genes involved in those processes suggests that the eggs of virgin fish are overloaded with transcripts related to translation, preparing the future embryo for intensive protein synthesis, which would require high energetic costs. It can be assumed that the identified DEGs originate from the small abnormal eggs of virgins, indicating that these eggs were probably not fully mature. In conclusion, reproductive experience plays a critical role in improving egg quality, which is also well reflected in the transcriptomic profile of eggs. The widespread upregulation of DEGs in virgin fish eggs may reflect an attempt to overcome intrinsic deficiencies, leading to lower developmental competence. Future research should focus on the specific functions of the identified DEGs to uncover their influence on egg quality in virgin fish. Comparative transcriptomic analysis of selected small abnormal and normal eggs from virgin fish is essential to verify the source of maternal transcript upregulation.

Funding: National Science Center, Poland (NCN) [grant number 2016/22/M/NZ9/00590].

[O31] New insights into morphological and macromolecular building of spermatogenic cells of smooth-hound sharks, *Mustelus mustelus*

Chemello, G.[1,2*], De Santis, L. J.[1], Giorgini, E.[1], Trotta, E.[1,2], Maradonna, F.[1,2], Carnevali, O.[1,2], and Gioacchini, G.[1,2]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy
[2]Istituto Nazionale Biostrutture e Biosistemi, Consorzio Interuniversitario (INBB), Rome, Italy

*Presenting author: g.chemello@univpm.it

Elasmobranchs have an ancestral reproductive system which offers insights into vertebrate reproductive evolution. Despite their unchanged design over 400 million years, they have evolved complex mechanisms ensuring reproductive success. However, human activities induced a significant decline in elasmobranch populations worldwide. In the Mediterranean basin, the smooth-hound shark (*Mustelus mustelus*) is one of the species that are considered vulnerable to human activities. Conservation efforts necessitate a thorough understanding of its reproductive strategy. This study focused on mature male specimens of smooth-hound shark caught in the Adriatic area and successively analyzed to provide, for the first time, a histological and macromolecular detailed description of testicular development in this species. Seven phases of the spermatogenesis process were identified together with cells' macromolecular characterization obtained using Fourier Transform InfraRed Imaging. Histological analysis showed structural and cellular features similar to those documented in the spermatocysts of other elasmobranch species. The examination of the evolution and migration of both germinative and Sertoli cells, at each phase, revealed their close connection. Furthermore, different expression levels of lipids, proteins, and phosphates (DNA) at each spermatogenesis stage were observed. This study provided a comprehensive overview of the morphological, structural, and molecular configuration of all stages characterizing the sperm development of the smooth-hound shark highlighting the unique characteristics that distinguish this species from other elasmobranchs. The information provided by this study are essential to better understand morphological and biochemical changes during spermatogenesis process of the smooth-hound shark. The exhaustive characterization of this process could represent a valid tool to recognize possible spermatogenesis impairment due to environmental changes. This aspect is crucial in terms of conservation, since the effects of overexploitation could be worsened by the more frequent environmental alterations which characterized the present era. Moreover, InfraRed Imaging represents an innovative tool to investigate cellular mechanisms during spermatogenesis that underlie essential processes for the survival and growth of a species.

[O32] *11ß-hydroxysteroid dehydrogenase type 2* silencing impairs zebrafish male reproduction

Tesoriere, A.[1], Terrin, F.[1], Castello, I.[1], Pagliarusco, T.[1], Locatello, L.[2], Martini, P.[3], Pavlidis, M.[4], Carnevali, O.[5], and Dalla Valle, L.[1*]

[1]Department of Biology, University of Padova, Padova, Italy
[2]Department BEOM, Stazione Zoologica Anton Dohrn, Fano Marine Center, Fano, Italy
[3]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
[4]Department of Biology, University of Crete, Crete, Greece
[5]Department of Environmental and Life Science, Polytechnic University Marche, Ancona, Italy

*Presenting author: luisa.dallavalle@unipd.it

In vertebrates, the 11ß-hydroxysteroid dehydrogenase type 2 (Hsd11b2) inactivates glucocorticoids (GCs), thus regulating the bioavailability of their active forms. The concentration of cortisol under stress response is primarily regulated by this enzyme, as evidenced by higher cortisol levels and a delay in returning to baseline steroid levels after stress induction in *hsd11b2* zebrafish mutants with respect to wild-type (WT) siblings. In Leydig cells, Hsd11b2 is also one of the enzymes responsible for the synthesis of 11-ketotestosterone (11-KT), the main active androgen in teleost. In agreement with all these evidence, *hsd11b2* mutants show impaired reproductive capabilities, especially in males. Since GCs are known to influence testicular functions, the aim of this work was to characterize how the lack of 11-KT and a possible higher local concentration of active GCs can significantly affect the fertility of mutant males. Although we did not find any clear difference in testis morphology between WT and mutant individuals, the area occupied by spermatozoa is slightly less extended in mutants. Further sperm quality analysis revealed no differences between groups in sperm velocity, motility, viability, and DNA fragmentation but emerged a significant reduction in total sperm count, thus corroborating the histological outcome. Despite *hsd11b2* mutants are almost unable to reproduce through conventional breeding, *in vitro* fertilization experiments confirmed that mutant sperm retains fertilizing ability. To gain further insights into the molecular pathways altered by the silencing of *hsd11b2*, a testis transcriptome analysis was performed. The study revealed that many up-regulated genes are involved in steroid biosynthetic process. The majority of down-regulated genes are, instead, involved in microtubule formation and transport, cilium assembly and organization, sperm flagellum composition and meiosis. Together, the considerable number of down-regulated genes and the similar level of their expression reduction, suggest that *hsd11b2* mutants could have a lower number of germ cells at the last maturation stages, confirming the important role of Hsd11b2 and 11-KT in germ cells maturation and male fertility. However, since these results cannot fully explain the reproductive impairment of these mutants, we will further proceed to examine a possible alteration of breeding behavior.

Funding: Partially funded by UoC and UniPD resources.

[O33] Single cell RNA-seq sheds light into the functional role of TGFß and BMP signaling in zebrafish germ cell populations

Ribeiro, A. O.[1], Nakajima, R. T.[1], Costa, D. F.[1], Rodrigues, M. S.[1], Marques, B. S.[1], and Nóbrega, R. H.[1*]

[1]Reproductive and Molecular Biology Group, Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

*Presenting author: rafael.nobrega@unesp.br

Single-cell RNA sequencing (scRNA-seq) allows the identification and characterization of cell populations throughout the transcriptional signature of each single cell analyzed. This revolutionary technology is especially useful to address the inherent complexity of tissues with a great variety of cell types, such as the testis. Here, we have used scRNA-seq assays to explore the cellular composition of zebrafish testes that went through spermatogenesis recovery after 17ß-estradiol-induced inhibition, aiming to shed light into processes occurring when undifferentiated spermatogonia undergo proliferation and differentiation. From the integrated transcriptomes of 19,626 cells, we identified clusters representing different testis cell populations, including two major clusters of spermatogonia, one of spermatocytes, and three of spermatids, along with groups of blood and immune cells. Interestingly, we found that the TGF-ß/BMP signaling related genes *ndr1*, *bambib*, *myca*, *mapk3*, and *id4*, along with the SMAD coding genes *smad1* and *smad9*, are highly expressed within the cluster that comprises the earliest generation of spermatogonia detected here. As the expression of *ddx4* decreases and *dazl* transcripts begin to accumulate, the expression of *smad2*, *smad5*, and *smad7* enhances, suggesting that these SMADs increase as the differentiation process runs. Since the TGF-ß superfamily plays important roles in the balance between germ cell proliferation and differentiation, we assessed the effects of DMH1 – a selective inhibitor of the BMP receptor activin receptor-like kinase 2 (ALK2) – and A83-01 – a TGFß pathway inhibitor that acts on the receptors ALK4, ALK5, and ALK7 – on the composition of different spermatogonial generations: type A undifferentiated (A_{und}), type A differentiated (A_{diff}), and type B spermatogonia. To achieve that, we analyzed the proportion of the area occupied by these three cell types in relation to the area covered by all the other testicular cells after 7 days of *ex vivo* culture of zebrafish testes with and without the ALK inhibitors. The surface area covered by A_{diff} spermatogonia decreased when BMP signaling was inhibited, pointing that the BMP pathway is a player in spermatogonial differentiation. However, TGFß and BMP signaling have opposite roles on cell proliferation and differentiation, since DMH1 treatment significantly decreased the area occupied by B spermatogonia – as well as the expression of *smad1* – while A83-01 exposure increased the surface covered by these germ cell types – as well the expressions of both *smad3a* and *smad3b*. Taken together, these results suggest that the TGF-ß subfamily is mainly involved in the maintenance of the undifferentiated state of spermatogonia, while BMP acts towards germ cell differentiation.

Funding: São Paulo Research Foundation (FAPESP) – grant numbers 21/06742-5, 22/10389-1, 22/10421-2, 23/13211-1 and 19/22997-3.

[O34] Oxytocin and relaxin signaling pathways are involved in the gonadotropic regulation of cell-cell interaction in Senegalese sole testis

López-Fortún, N.[1,2*], Roig, J. V.[3], Giménez, I.[3], Cerdà, J.[1,2], and Chauvigné, F.[1,2]
[1]Institute of Marine Sciences, CSIC, 08003, Barcelona, Spain
[2]Institute of Biotechnology and Biomedicine (IBB), Universitat Autonoma de Barcelona, 08193 Bellaterra (Barcelona), Spain
[3]Rara Avis Biotec, S.L., 46002 Valencia, Spain

*Presenting author: noelialopez@icm.csic.es | *Student*

Our understanding of the semi-cystic spermatogenesis in teleosts, such as the Senegalese sole (*Solea senegalensis*), and particularly of the process by which spermatids are released into the lumen of the seminiferous tubules where they differentiate to spermatozoa (spermiogenesis), is very limited. In sole, we have previously shown that the luteinizing hormone (Lh) can penetrate the blood-testis barrier to reach the free spermatids expressing the Lh receptor, thereby stimulating spermiogenesis. However, the cellular mechanisms involved in this process are unknown. The present study therefore aimed to investigate whether gonadotropins can regulate across the blood-testis barrier function in Senegalese sole. To this end, we conducted a six-week trial treating male sole with 6 µg/kg recombinant folliclestimulating hormone (rFsh) or Lh (rLh), or saline (controls), once a week. Following the treatments, we performed a transcriptomic analysis employing RNA-seq to identify differentially expressed genes (DEGs) among the groups. We found that rFsh and rLh significantly regulated 717 and 1316 genes, respectively, with 357 DEGs common to both treatments. Pathway analysis revealed that both gonadotropins influenced the expression of the oxytocin (OXT) and relaxin (RLN) signaling pathways, as well as of cell-cell interaction networks. *In situ* hybridization and immunohistochemistry indicated that the OXT and RLN receptors are primarily expressed in Sertoli cells and spermatids, whereas cell-cell interaction related mRNAs previously identified in the RNA-seq analysis as being down-regulated by rFSh or rLh, including Ctnnb1, Pard3, Gja3, Tjp2, Cldn4, Tmem47 and Itbg1, were detected in either spermatids or Sertoli cells. To confirm these observations, testicular explants were incubated *in vitro* with OXT, RLN or their corresponding receptor antagonists (L-371 and AT001, respectively), in combination with rFsh or rLh. Furher qPCR analysis indicated that both rFsh and rLh down-regulated cell-cell interaction related transcripts, the action of rLh being more potent and predominantly through the inhibition of the OXT and RLN signaling pathways. These findings suggest that gonadotropins, and particularly Lh, can repress cell-cell interaction mechanisms between Sertoli cells and Sertoli cells-spermatids, thereby enhancing the permeability of the blood-testis barrier and the release of spermatids to facilitate luminal spermiogenesis.

Funding: Supported by the Spanish Ministry of Science, Innovation and Universities (PID2021-126128OB-I00 to F.C.; PRE2022-101651 to N.L.F. and CEX2019-000928-S to ICM).

[O35] Loss of Fshr prevents testicular maturation in Atlantic salmon

Andersson, E.[1], Schulz, R. W.[*1,2], Almeida, F.[3], Kleppe, L.[1], Skaftnesmo, K. O.[1], Kjærner-Semb, E.[1], Crespo, D.[1], Fjelldal, P. G.[1], Hansen, T. J.[1], Norberg, B.[1], Edvardsen, R. B.[1], and Wargelius, A.[1]

[1]Institute of Marine Research, Bergen, Norway
[2]Science Faculty, Department Biology, Utrecht University, Utrecht, The Netherlands
[3]Embrapa Amazônia Ocidental, Manaus, Amazonas, Brazil

*Presenting author: r.w.schulz@uu.nl

Early puberty poses significant challenges for male Atlantic salmon in aquaculture due to its negative impact on growth and welfare. The regulation of puberty involves follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh) and their respective receptors, Fshr and Lhcgr. Mice lacking a functional FSHR are fertile, but testis size, sperm production and motility are reduced. However, medaka and zebrafish *fshr* mutants exhibit normal testis size and fertility; the start of puberty is delayed in *fshr* mutant zebrafish, but Lh/Lhcgr signaling eventually seems to fully rescue puberty/fertility. In salmonid fish, Fsh is the only gonadotropin circulating during the initiation of puberty and much of the ensuing testis growth period. To test the hypothesis that Fshr signaling is required to trigger male puberty in salmon, we used CRISPR-Cas9 to mutate the *fshr* gene and obtained a high prevalence of *fshr* mutations at the target site. 17% of the crispants (F0 generation) remained immature and 35% showed a testis development deviating from wild-type (wt) controls, of which 6% remained immature and 94% matured normally. Crossing out F0 crispants to each other produced a viable F1 generation showing different mutations, including bi-allelic frameshift (*fshr−/−*) and in-frame mutations (*fshrif/if*), the latter missing one amino acid. With a single exception, all wt males matured while all *fshr−/−* males remained immature. *fshr−/−* testes contained type A spermatogonia but no further differentiated germ cell types; androgen plasma levels remained low. Also, pituitary transcript levels of *gnrhr2bba* and *lhb*, but not of *fshb*, remained low in *fshr−/−* mutants. Interestingly, the loss of a single amino acid resulted in more than half of the *fshrif/if* mutants showing no or a delayed maturation. In conclusion, Atlantic salmon so far are unique among vertebrates in that Fshr function is required for male fertility, offering new opportunities to control precocious male puberty/fertility.

Funding: Grants nos. 267610 (Salmosterile), 245979 (Sterwell), 254870 (Postsmoltmat), and 336213 (Salster) from the Norwegian Research Council.

Session AQUA [Chair: Dr. Juan F. Asturiano and Dr. Alicia Felip]

[O36] Gonadotropin gene therapy in fish: from basic research to practical applications in aquaculture

Zapater, C.[1*], Cruz-Castellón, C.[1,2], Mazón, M. J.[1], Morini, M.[2], França, T. S.[2], Pérez, L.[2], Asturiano, J. F.[2], and Gómez, A.[1]

[1]Grupo de Fisiología de la Reproducción de Peces, Instituto de Acuicultura de Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellón, Spain

[2]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València, Valencia, Spain

*Presenting author: cinta.zapater@csic.es

Gonadotropins (GTHs), follicle-stimulating hormone and luteinizing hormone, play central roles in vertebrate reproduction. In most fish species their functions during gametogenesis are gradually being elucidated, though specific roles remain not well defined. The use of recombinant GTHs offers a unique tool for studying the regulation of gametogenesis and addressing reproductive issues observed in captive fish species. However, the high production costs of recombinant GTHs make them unsustainable for the aquaculture sector.

As an alternative, this study evaluates the use of GTHs gene therapy to induce and study spermatogenesis in fish. European sea bass was selected for validating this technique due to the already existing knowledge on its reproductive patterns and the availability of various tools for physiological studies. GTHs somatic gene transfer resulted in higher and more sustained levels of GTHs in plasma compared to direct injection of recombinant hormones. This treatment induced a physiological effect on the injected fish similar to that of direct injection of recombinant GTHs, increasing steroid levels, activating spermatogenesis, and enhancing both sperm production and quality. This findings indicate that GTHs somatic gene transfer could serve as an effective gene therapy for addressing reproductive dysfunctions in certain fish species.

The European eel was used to further investigate the potential of somatic gene transfer to improve reproduction, as its maturation in captivity typically relies on the use of recombinant proteins. Three experimental groups were established: (i) Injected with a plasmid coding for an eel single-chain follicle-stimulating hormone (scFsh), (ii) injected with both eel scFsh and eel single-chain luteinizing hormone (scLh) plasmids, and (iii) a control group injected with an empty plasmid. To assess the effectiveness of the injections, all groups received a small amount of sea bass scFsh plasmid, which can be monitored in plasma and does not have a functional effect in eel. The results demonstrated that GTHs plasmid gene therapy could trigger steroidogenesis and spermatogenesis in male European eels, achieving spermiation in some cases. The combination of Fsh and Lh plasmids produced a more pronounced effect with less variability. In conclusion, compared to other hormonal treatments, the low production cost and high efficiency of GTHs gene therapy highlight its potential to contribute to the basic study of gametogenesis in fish and to solve reproductive dysfunctions in aquaculture.

Funding: MCIN with funding from NextGenerationEU (PRTR-C17.I1) and GVA (THINKINAZUL/2021/012; THINKINAZUL/2021/042). GVA (CIAICO/2022/002). Fundación Carolina/UPV supported C.C.

[O37] Effects of multiple hormonal stimulation and stripping during out-of-spawning season on sperm quality of common carp *Cyprinus carpio*

Siddique, M. A. M., Shazada, N. E., Zhang, S., Linhart, O., and Boryshpolets, S.
University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic

*Presenting author: siddique@jcu.cz

Without hormonal stimulation, the quantity and quality of common carp milt varies significantly by multiple stripping during the spawning season. However, the effects of multiple hormonal stimulations and continuous stripping during the out-of-spawning season are unclear. Thus, this study aimed to investigate motility parameters and seminal plasma compositions of common carp sperm during out-of-spawning continuous stripping stimulated by multiple hormonal injections. Five mature males cultured in RAS at a stable temperature of 21 °C with 1-2 Kg body weight were used in this study. Fish were treated monthly (August-October) with carp pituitary at 1.5 mg/kg (b.w.) dissolved in 0.9% (w/v) NaCl solution 24 h before milt collection. Sperm concentrations, motility parameters and seminal plasma composition were determined each month. The mean sperm concentration ranged from 17.55 to 20.19 × 10^9 mL^{-1} collected during the out-of-spawning season (August-September) and did not significantly vary among these sampling months ($F = 2.352$, $p = 0.1374$). The repeated measures ANOVA showed significant effects of sampling months ($F = 25.24$, $p = 0.0001$) and males ($F = 7.50$, $p = 0.0001$) on the motility parameters and seminal plasma compositions, but their interaction effects were not significant ($F = 1.31$, $p = 0.079$). When the saturated model decomposed to determine the effects of multiple stimulations and stripping during three consecutive months of the out-of-spawning season, we found a significant difference in spermatozoa motility ($F = 10.51$, $p = 0.0001$) and pH of the seminal plasma ($F = 90.00$, $p = 0.0001$), but did not differ in curve linear velocity (VCL), straight-line velocity (VSL), and ionic compositions (Na^+, K^+, Cl^-, Ca^2, total protein, and osmolality of the seminal plasma ($p > 0.05$). The mean spermatozoa motility in August (88.51 to 94.05%) and September (89.88 to 96.00%) was significantly higher than in October (83.99 to 89.58%), but spermatozoa motility was >80% in all males for the three consecutive months. Again, we decomposed the saturated model to estimate the male effects on motility parameters of fresh sperm and seminal plasma composition. No significant male difference was observed in spermatozoa motility, VLC, VSL, or the pH of the seminal plasma ($p > 0.05$). A significant change was detected in Na^+, K^+, Cl^-, Ca^2, total protein, and osmolality in the seminal plasma of different males ($p < 0.05$). The present study revealed that multiple hormonal stimulation and continuous stripping did not suppress the sperm quality parameters of common carp, even during the out-of-reproductive period. Thus, the same males can be stimulated and stripped throughout the year for hatchery use.

Funding: The Ministry of Education, Youth and Sports of the Czech Republic - project "CENAKVA" [No. LM2018099].

[O38] The effect of visible light on early embryonic development in trout

Gao, L. and Pšenička, M.
Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic

Presenting author: lgao@frov.jcu.cz | *Student*

INTRODUCTION

Trout is an economically important fish, and its early embryonic development process directly affects the quality and productivity of adult fish. Light, as an important environmental factor, plays a crucial role in trout embryonic development. The early embryonic development stages of trout are particularly sensitive to light, trout embryos die when expose to light for more than a few minutes. However, the mechanism by which light affects early embryonic development in trout remains unclear. Mitochondria have been shown to have optical characteristics and absorb short wavelength light, which is considered harmful and associated with reduced function. The primary objective of this study was to explore the effects of various wavelengths of visible light on the early embryonic development of trout. We first tested the effect of light on trout embryos and then examined its impact on isolated mitochondria from these embryos, comparing them with mitochondria from other tissues of adult trout. Thus provide a scientific foundation for optimizing light management in trout aquaculture.

METHODS

Trout eggs and embryos were incubated at 12°C in an incubator. Mitochondria were isolated from different tissues and stored on ice for subsequent use. The experiments were conducted as follows: First, to determine the effect of visible light on the early embryonic development, 3-12 days post-fertilization (dpf) embryos were exposed to 415 nm, 475 nm, 515 nm, 550 nm and 590 nm light, respectively. Then, mitochondria from 3-12 dpf embryo were isolated and exposed to light that was harmful to the embryo (415nm) and control light that has no adverse effect (590 nm). Additionally, to test whether mitochondria from different tissues of trout and embryos and mitochondria from embryos of different species are sensitive to 415 nm light, mitochondria from trout egg, sperm, muscle and liver, as well as zebrafish embryos and embryonic mitochondria, were exposed to 415 nm and 590 nm light, respectively. Three replications of 10 embryos were performed under each light condition with a light intensity of 100 W m^{-2}. Each experiment had another control group with the same conditions but without light exposure. Finally, the viability of embryos and mitochondria after exposure was examined in each group.

RESULTS & DISCUSSION

We found that only 415 nm of visible light is harmful to trout embryos, and only to 3-9 dpf embryos. Mitochondria isolated from eggs and 3-9 dpf embryos were also sensitive to 415 nm light. However, mitochondria from other tissues of trout and zebrafish embryos, as well as zebrafish embryos, were insensitive to visible light at all. Half of 12dpf trout embryos were at the eyed stage. This implies that trout embryos are sensitive to 415nm light only before the eyed stage.

[O39] Using algae blend and micronutrients supplementation in breeders' diet to modulate gamete quality: the example of turbot

Oliveira, C. C. V.[1*], Marrero, C.[1], Parente, P.[1], Ramos-Júdez, S.[2], Fatsini, E.[1], Félix, F.[1], Duarte, D.[1], Beirão, J.[3], Medina, D.[4], Castro, C.[4], and Cabrita, E.[1]

[1]CCMAR, University of Algarve, Campus de Gambelas, ed. 7, 8005-139 Faro, Portugal
[2]S2AQUAcoLAB, Av. Parque Natural da Ria Formosa, s/n, 8700-194 Olhão, Portugal
[3]Nord University, Faculty of Biosciences and Aquaculture, Bodø, Norway
[4]FLATLANTIC, Rua do Aceiro s/n, 3070-732 Praia de Mira, Portugal

*Presenting author: ccoliveira@ualg.pt

Turbot, *Scophthalmus maximus*, is a highly appreciated flatfish species, produced in southern and northern European countries, still facing production challenges, namely in terms of reproduction in captivity. Larvae are obtained by artificial fertilization; however, gamete quality and availability are variable. Breeders' nutrition can be an effective approach for improving and standardizing gamete quality and maturation success in this species. The incorporation of micro- and macroalgae in the diet has previously shown to ameliorate reproductive traits in aquaculture species, besides being sustainable promising alternative ingredients. In this study the combined effect of an algae blend (*Spirulina platensis* and *Laminaria digitata*) and micronutrients (astaxanthin, vitamin C, and vitamin E) supplementation in breeders' diet was evaluated in turbot's sperm traits. Four captive-reared turbot broodstocks held at the company Flatlantic® were used (mean weight 6.08 kg), reared in 15 m3 tanks at a constant water temperature all year round (14.0 ± 0.5 °C), and under a simulated natural photoperiod. Each two tanks were fed a different diet: the regular commercial feed by Sparos Lda. (CONTROL) and the same diet supplemented with 5% Spirulina and 1% laminaria, an iodine-rich macroalgae, and fortified with nutrients such as astaxanthin, vitamin C, and vitamin E (ALGAE). Sperm samples were collected at two moments coinciding with the peak of the reproductive season for each broodstock, to evaluate the following sperm quality parameters: sperm motility and cell concentration (CASA system), lipid peroxidation (MDA determination), DNA fragmentation (Comet assay), cell viability and Reactive Oxygen Species (ROS) (flow cytometer). Also, blood and seminal plasma were collected to determine sex steroids levels, oxidative stress enzymes (Glutathione reductase, GSR, Glutathione peroxidase, GPX and Superoxide dismutase, SOD) and TAS (Total antioxidant status). The results revealed that the ALGAE diet increased sperm velocity, decreased DNA fragmentation, and seemed to advance gonadal maturation in this group of fish. In other quality parameters such as cell viability, lipid peroxidation, caspases and ROS the benefits were not so evident, and the differences encountered were more related to the moment of the reproductive cycle. Algae supplementation in breeders' feed seems to be a promising approach to modulate sperm traits, although more research is needed to study other sources.

Funding: "Blue Growth Program (#4)" PT-INNOVATION-0080 (EEA grants project-BREEDFLAT) and FCT (UIDP/04326/2020, LA/P/0101/2020 and contract 57/2016/CP1361/CT0007).

[O40] Mechanisms of potassium signaling in fish spermatozoa motility

Rashed, M.* and Bondarenko, O.
Laboratory of Reproductive Physiology, Research Institute of Fish Culture and Hydrobiology, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic

*Presenting author: mrashed@frov.jcu.cz | *Student*

Enhancing the success of fertilization of eggs with sperm requires extensive physiological studies of both eggs and sperm. The motility of sperm is one of the crucial phenomena for successful fertilization that enables sperm to reach the egg's surface. The activation of sperm motility depends on several physiological aspects, and changes in ion concentration are one of the most important features of them. Studies have confirmed that the change in osmolality followed by K^+ efflux is the first stimulus for the initiation of motility of many fish species. Transmembrane K^+ efflux is responsible for membrane hyperpolarization and acts as the first trigger for the initiation of spermatozoa motility. Transportation of K^+ is carried out by several types of K^+ channels, among them SLO1, SLO3 and CNGK play a significant role in the regulation of spermatozoa motility in various species. SLO1 and SLO3 genes have been extensively studied in the spermatozoa of humans and other vertebrates and found to be responsible for sperm capacitation and acrosomal reaction (AR). K+-selective cyclic nucleotide-gated (CNGK) channels have been detected in the sperm of sea urchins and zebrafish. However, there is no conclusive study showing the functional presence of those channels in the spermatozoa of different fish species. Besides, K^+ channels are very sensitive to some chemicals, potentially incorporated into the aquatic environment by industrial (some heavy metals), agricultural (inhibitors of bacterial growth in the commercial ponds) and medical (various types of drugs that control the activity of K^+ channels in the human body) activities. A study using exploratory genomics has postulated that there might be a great chance of the presence of the SLO3 gene in fish spermatozoa. In the present study, we have developed a hypothesis that those three channels might be present in spermatozoa of different fish species. For this, we have prepared a phylogenetic tree using amino acid sequences of those channels of different groups of animals. Based on our results, we may predict the presence of SLO1 in carps, trouts, pikes, perches, sturgeons, and eels; SLO3 in trouts, pikes, perches, and gars; CNGK in trouts, sturgeons, carps, gars and eels. In the future, we are planning to confirm the presence of SLO and CNGK channels in the spermatozoa of some of those fishes. Moreover, we intend to characterize the roles of these channels in sperm motility and fertilization and specify concentrations of contaminants that can threaten natural reproduction, hindering the motility of spermatozoa in those species.

Funding: This study was financially supported by the Ministry Institute of Education, Youth and Sports of the Czech Republic, CENAKVA (LM2023038), Biodiversity (CZ.02.1.01./0.0/0.0/16_025/0007370), Grant Agency of the University of South Bohemia in Ceske Budejovice (GAJU 107/2022/Z).

[O41] Sperm motility of freshwater species- carp trout and sturgeon in viscous environments

Mueni, L. M.*, Kholodnyy, V., Sotnikov, A., and Boryshpolets, S.
Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, Vodňany 389 25, Czech Republic

*Presenting author: lmueni@frov.jcu.cz | *Student*

Spermatozoa motility is crucial for fertilization success and is related to sperm quality and fertilizing capability. However, the interactions between the spermatozoa and the environment surrounding the gamete during their collision are largely overlooked. These environments are unique in various species, often include some reproductive fluid (ovarian/uterine/coelomic), and are characterized by higher viscosity compared to water. Nonetheless, little is known about these viscous environments in external fertilization, even though ovarian fluid has been shown to affect activation, longevity, swimming performance and trajectory of spermatozoa. Hence, we aim to understand how various viscosities may impact spermatozoa performance in several freshwater species - common carp, sterlet, and rainbow trout characterized by short and quick motility. The spermatozoa from the species are also characterized by different physiology and structure. Sterlet spermatozoa have elongated cylindrical heads, slightly oval shapes in rainbow trout, and more round shapes in common carp. This insight is relevant for comprehending the mechanisms of adaptation of fish reproduction to diverse environments. This study used solutions of different viscosities created by adding methylcellulose (MC) at different percentages to activate the motility of spermatozoa. We analyzed different parameters of sperm motility, such as velocity, linearity, and wobble of spermatozoa trajectories, using CASA and the flagellum beating parameters, such as wave propagation speed and shape, using high-speed video microscopy. As the viscosity increased, the velocity and motility percentage of the studied spermatozoa decreased. We also observed the planar motion of the flagellum in low viscosity. In contrast, in high-viscosity media, some spermatozoa could progress and produce helical waves in all three studied species. Other parameters of spermatozoa motility (linearity, wobble, straight-line velocity, etc.) were also affected by viscosity, accompanied by flagellar wave compression in high-viscosity media. As a result, more circular motility patterns were observed. The study indicates that the viscosity of the swimming media affects the motility and performance of these three freshwater species, although further detailed research is needed.

Funding: the Ministry of Education, Youth and Sports of the Czech Republic - project "CENAKVA" [No. LM2018099].

[O42] Performance of offspring salmon produced using fresh and cryopreserved semen exposed to multiple stresses (thermal stress and bacterial challenge)

Erraud, A.[1*], Cornet, V.[1], Lambert, J.[1], Neus, Y.[2], and Kestemont, P.[1]
[1]URBE (ILEE), University of Namur, Belgium
[2]Conservatoire du Saumon Mosan (CoSMos), Erezée, Belgium

*Presenting author: alexandre.erraud@unamur.be

The impact of sperm cryopreservation on offspring performance in fish is a topic of ongoing debate. Depending on the source, cryopreservation may result in an artificial selection of spermatozoa, which may have positive or negative effects on the average phenotypes of the offspring. Sperm cryopreservation may affect the genetic information transmitted to the zygote, potentially impacting the development of the offspring. Maternal effects are likely to dominate in the early embryonic stages, with other effects expressed later. The aim of this experiment was to test the effects of two stressors (i.e. river temperature and bacterial stress) on *Salmo salar* fry. Fifteen days after hatching, organisms derived from fresh and cryopreserved sperm were subjected to daily temperature fluctuations of varying degrees (i.e. 9°C; 7 - 12°C and 3 - 15°C) over a period of 11 days, followed by a 24-hour bacterial challenge (*Aeromonas salmonicida*) and a 5-day depuration period. Subsequently, biometric and transcriptomic qPCR analyses were conducted on the various experimental conditions. Organisms from cryopreserved semen consumed greater amounts of vitelline reserves than those obtained from fresh semen, mainly due to high daily temperature fluctuations ranging from 3 to 15°C. Transcriptomic results support these observations, as organisms produced by cryopreserved semen showed over-expression of genes linked to antioxidant defenses and energy metabolism. The temperature increase led to an increase in intracellular ROS and, consequently, an over-consumption of energy to cope with this stressor. It is probable that organisms from cryopreserved semen have a narrower optimal temperature range than organisms from fresh sperm. Additionally, exposure to bacterial challenge has demonstrated that organisms from the cryopreserved group can defend themselves against pathogens only if they have not previously been exposed to thermal stress. Research has demonstrated that the impact of the bacterial challenge has long-term consequences on transcript levels, resulting in decreased antioxidant and innate immune defense capabilities five days after the challenge. In conclusion, it appears that sperm cryopreservation results in a decrease of organisms' capacity to respond to various stressors. A decrease in the ability to cope with this type of stressor may result in higher mortality rates in organisms derived from cryopreserved semen compared to those derived from fresh semen. Further studies could be conducted over a longer period with organisms such as parr to evaluate the impact of sperm cryopreservation on the later development of organisms, particularly through a study in their natural environment.

Funding: This study was supported by the project Meuse Salmon funded by the Public Service of Wallonia, Belgium(Saumon Meuse VISA 19/15173).

Plenary session

[K3] Environmental conditions during early development and their effects on the fish epigenome

Piferrer, F.
Institute of Marine Sciences (ICM), Spanish National Research Council (CSIC), Barcelona, Spain

Presenting author: piferrer@icm.csic.es

In this talk, I will begin by discussing some concepts of epigenetics, with a focus on fish. We will explore the various internal and external (environmental) factors that can modify the fish epigenome, emphasizing DNA methylation, perhaps the most extensively studied of the primary epigenetic mechanisms. We will also identify three critical windows during which external factors can significantly influence the fish epigenome. Special attention will be given to reprogramming events during early development, highlighting the diversity of identified patterns in fish compared to mammals. Indeed, transgenerational epigenetic inheritance is a prominent topic in biology, and fish present a unique opportunity among vertebrates for research in this area. We will discuss how early environmental conditions can lead to changes with lifelong consequences, impacting individual growth and gonadal development as well as population-level factors such as the sex ratio. After showing the importance of epigenetic modifications also in the early stages of domestication, we will discuss the identification of metastable epialleles and pure epialleles, illustrating what sort of question they can help to address. We will finish explaining how to incorporate epigenetic selection alongside genetic selection programs and introduce a novel approach involving epigenetic markers for genome screening. An example with potential applications in finfish farming will be provided to demonstrate these concepts.

Funding: Agencia Española de Investigación, projects EPIMARK (AGL2016-78710-R), EPIPURE (PID2019-108888RB-I00) and EPIBASE (PID2022-139096OB-I00).

9TH INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

POSTER SESSIONS

Odd numbers will be defended on Poster session 1 on Tuesday 16.07.24

Even numbers will be defended on Poster session 2 on Wednesday 17.07.24

Session GERM

[P1] Partial gonadectomy: A new approach to obtain germline stem cells for transplantation while preserving the donor

Nayak, R.*, Pšenička, M., and Franěk, R.
Faculty of Fisheries and Protection of Waters, USB, Vodnany, Czech Republic

Presenting author: rnayak@frov.jcu.cz | *Student*

Surrogacy involves the transplantation of germline stem cells from the donor into the recipient of interest to produce germline chimera. It offers many scopes of restoration of lines from cryopreserved germ stem cells and faster propagation of species with a long maturation time with the help of a surrogate. However, studies reported to date always involved donor sacrificing prior to germ cell collection. Such a practice can significantly hamper the decision of whether surrogacy should be employed as a propagation method for valuable species. Here, we show a non-lethal approach to obtain germline stem cells from adult zebrafish males. We confirm that non-lethally collected spermatogonial cells successfully colonized the recipient gonad and gave rise to donor-derived sperm and, subsequently, to viable progeny. Testicular tissue donors showed no mortality after partial gonadectomy. Moreover, their reproductive characteristics were not compromised, and gonads were regenerated. Turquoise killifish males showed no significant differences in their reproductive output compared to the controls post partial gonadectomy. Our study presents robust evidence that surrogate reproduction can be leveraged by partial gonadectomy in small-bodied fish (~0.5 g) while keeping the donor alive and preserving its breeding value. Therefore, we expect that its application in large aquaculture species is feasible and will serve as a viable alternative to the commonly used lethal approach for donor cell collection. Afterward, gonadal tissue donors can still be involved in the breeding cycle while their germ cells are safely stored in liquid nitrogen or propagated through surrogates.

[P2] Assessing recipient suitability for European eel (*Anguilla anguilla*) spermatogonia xenotransplantation

Blanes-García, M.[1], Marinović, Z.[2], Morini, M.[1], Šćekić, I.[2], Lujić, J.[2], Ferrão, L.[1], Urbányi, B.[2], Horváth, Á.[2], Vergnet, A.[3], and Asturiano, J. F.[1*]

[1]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València. Valencia, Spain
[2]Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences. Gödöllő, Hungary
[3]MARBEC Université de Montpellier, CNRS, Ifremer, IRD, INRAE, Palavas-les-Flots, France

*Presenting author: jfastu@dca.upv.es

The European eel is a commercial species with limiting challenges to achieve sexual maturation in captivity. This study explores the surrogate broodstock technology as an alternative method to the traditional long hormonal treatments, by using three different recipient species: common carp (*Cyprinus carpio*), European sea bass (*Dicentrarchus labrax*) and zebrafish (*Danio rerio*). Spermatogonia from immature European eel testes was obtained by dissociation of fresh or cryopreserved tissue, employing an enzymatic solution. Prior to transplantation, the cells were labelled with fluorescent marker PKH26. Eel spermatogonia isolated from fresh tissue were xenotransplanted into wild-type zebrafish larvae and triploid European sea bass larvae, while spermatogonia obtained from cryopreserved testis were xenotransplanted into transgenic *vasa::egfp* zebrafish larvae and germ-cell depleted by *dnd1*-knockdown common carp. After 1.5 months post-transplantation (mpt), donor fluorescent cells were only detected in common carp gonads, but at 6 mpt, qPCR did not reveal European eel specific-gene expression of *vasa1*, *vasa2* or *dnd1* in the gonads. Moreover, donor fluorescent cells were not detected neither in European sea bass or zebrafish at 1.5 mpt, and qPCR analysis did not reveal European eel gene expression at 1.5 or 6 mpt. The use of different species as surrogate recipients for European eel spermatogonia suggests that spermatogonia migration mechanisms are not well-conserved in zebrafish and European sea bass. On the other hand, common carp apparently does not possess an appropriate gonadal microenvironment for the development and proliferation of European eel spermatogonia. Phylogenetically closer species might be more suitable for surrogate European eel production.

Funding: MCIU (EELGONIA; RTI2018-096413-B-100), NextGenerationEU (PRTR-C17.I1), GVA (THINKINAZUL/2021/012, SEASPERM), EU H2020 (AQUAEXCEL, 871108), NKFIH K138425 and FK 142933, Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences. MBG had PhD contract from UPV (PAID-01-20).

[P3] Establishment of 3D testicular organoid system as a novel tool to study spermatogenesis in fish

Ferreira, J.[1*], Nóbrega, R. H.[1,2], and Pšenička, M.[1]
[1]Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, 389 25 Vodňany, Czech Republic
[2]Reproductive and Molecular Biology Group, São Paulo State University, Botucatu 18618-970, Brazil

*Presenting author: psenicka@frov.jcu.cz | *Student*

INTRODUCTION

Spermatogenesis is a stem-cell driven system which requires a complex multicellular interaction and intricate signaling. The established 2D culture systems, such as organotypic cultures, do not reflect the complex cell-cell interaction and signaling as observed *in vivo*. Recently, the establishment of scaffold-free or scaffold-based testis cell culture systems would provide a 3D microenvironment to support testis cells growth and development, facilitating the *de novo* testis organogenesis and functioning. This artificial system would permit a more detailed investigation of physiological testicular functions, including the interactions between germline stem cells and somatic cells, as well as mechanisms of involved in male puberty or infertility. The current study aimed to develop a scaffold-based and scaffold-free testis culture system in fish using sterlet sturgeon (*Acipenser ruthenus*) as model species.

METHODS

We tested two approaches to generate 3D testis culture; the first one by encapsulating the testicular cells inside a hydrogel (scaffold-based) that mimics the biological and mechanical proprieties of the extracellular matrix, and the second, by employing a centrifugal forced aggregation with microwells which generate a large number of size and composition-controlled spheroids (scaffold-free). For the first approach, we used a commercially available hydrogel, named Vitrogel ORGANOID (The Well Bioscience), a synthetic hydrogel that mimics the extracellular matrix, and has four formulations (Vitrogel 1,2,3 and 4) with different stiffness (50 to 300Pa). In the second approach, we used Aggrewell (StemCell Technologies) plates which can generate up to 1200 spheroids/well. Testes from 2 years old sterlet sturgeon were enzymatically dissociated and the testicular cells were either encapsulated in four different types of Vitrogel and incubated in specific medium for 20 days. Testicular cells were also placed into Aggrewell plates, centrifuged, and incubated for 7 days in a specific medium. Cell aggregates were observed under microscope, and some were processed for histological analysis.

RESULTS & DISCUSSION

Our results showed that testicular cells were able to form aggregates in all types of Vitrogel, although round shaped aggregates could be observed only in Vitrogel 1. In the other types of Vitrogel, cell aggregates formed disorganized, grape shaped structures which probably reflects the influence of different stiffness on the cell structure. Focusing on Vitrogel 1, we observed that cell aggregates formed organized structures with an epithelium at the periphery resembling most likely testicular cord as found *in vivo*. During culture period, cell aggregates increased in size and cellular density, although the growth ratio remained stable from day 17. We also observed cellular buds which also increased in size and cellular density during the period of culture. Histological analysis confirmed that cell aggregates showed similar structure of a germinal compartment as observed *in vivo*, although cellular markers are needed for a better cellular characterization. We also demonstrated that that Aggrewell was more efficient in generating aggregates in shorter time (7 days), with higher number, large size and more uniform shape. Both systems support 3D testicular cell culture, but cellular complexity and development need to be further investigated as well as their potential applications to aquaculture.

The project received funding from FAPESP-GACR (21/03739-3) and Aquaexcel 3.0 (2020-2025).

[P4] Dimorphic expression of sex-related genes suggests differential PGC proliferation between the two sexes of African catfish (*Clarias gariepinus*) juveniles

Balogh, R. E.[1*], Csorbai, B.[1], Guti, Cs.[1], Horváth, Á.[1], Urbányi, B.[1], Orbán, L.[2], and Kovács, B.[1]

[1]Institute of Aquaculture and Environmental Safety, Szent István Campus, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary
[2]Frontline Fish Genomics Research Group, Department of Applied Fish Biology, Institute of Aquaculture and Environmental Safety, Georgikon Campus, Hungarian University of Agriculture and Life Sciences, Keszthely, Hungary

*Presenting author: balogh.reka.eniko@uni-mate.hu

We mapped a previously isolated male-specific DNA marker (CgaY1) to the recently published African catfish genome sequence (Nguinkal et al., 2023) and investigated the early expression profile of proximate genes (*mark2*, *pten*, *amhr2*). Juvenile specimens were dissected into three segments (head, trunk and tail) under a stereomicroscope at 10, 15, 20, 30, and 40 days post-hatching (dph). Molecular sexing was conducted using the CgaY1 marker on DNA isolated from the fins, while gene expression analysis was performed on heads and trunks using RT-qPCR. We observed higher expression of *pten* in males at 20 dph in heads, persisting from 30 dph in both heads and trunks. Similarly, *amhr2* mRNA levels were elevated from 20 dph in both heads and trunks of males. Both *pten* and *amhr2* have been implicated earlier in the inhibition of PGC proliferation across mammals, fish, and cell cultures (Kimura et al., 2003; Adolfi et al., 2019). Notably, in several fish species (e.g., Japanese medaka, Nile tilapia, zebrafish), one of the initial signs of gonad differentiation is the increased number of PGCs in females, followed by somatic cell differentiation. Our findings suggest that similar events might occur in African catfish, potentially governed by signals originating not only from the gonads but also from the brain. To our knowledge, this study is the first investigation of sexually dimorphic early gene expression in African catfish utilizing molecular sexing with a sex-specific marker prior to the first signs of phenotypic sexual differences.

Funding: KKP 140353 to LO, MATE-K/3037-7/2023, NKIFH K138425, Horizont 2020 No 818036, 2017-2.3.3-TÉT-VN-2017-00004, GINOP Plusz 2.1.1.-21-2022-00100.

[P5] Tracking the germinal vesicle during *in vitro* maturation of transgenic *ddx4*:EGFP zebrafish oocytes

Kitanović, N.*, Marinović, Z., Csenki, Z. and Horváth, Á.
Hungarian University of Agriculture and Life Sciences (MATE), Institute of Aquaculture and Environmental Safety; P. Karoly 1., 2100, Gödöllő - Hungary

*Presenting author: kitanovic.nevena@uni-mate.hu | *Student*

During maturation, postvitellogenic ovarian follicles undergo considerable morphological changes that are used as markers for their progress *in vitro*. Cytoplasmic events, such as yolk reorganization and hydration—seen as the increase in ooplasm translucency and follicle size—can be easily scored under a light stereo microscope. However, the accompanying migration and breakdown of the germinal vesicle (GVBD) are predominantly visualized by precipitating nuclear components through "acid clearing" of the follicle, which renders it unusable for further study. As GVBD is the main indicator of meiosis resumption and developmental potential of the matured egg, here we report the use of follicles isolated from Tg(*ddx4:ddx4-EGFP*) zebrafish (*Danio rerio*) line to non-invasively monitor this process *in vitro*. Fully grown ovarian follicles (opaque, Ø 730±20 µm) were isolated from wild-type AB and transgenic zebrafish. Incubations were done in 90% Leibovitz L-15 medium, supplemented with antibiotics, 0.1% Bovine Serum Albumin and 15 mM HEPES (pH 7.5-9.0), with the addition of following hormones: 17α,0ß-dihydroxy-4-pregnen-3-one (DHP; 10-1000 ng/ml), human chorionic gonadotropin (hCG; 2-5 IU/ml) and prostaglandins (PGs; 1-10 µg/ml), alone or in combination. Maturation was evaluated by scoring ooplasm translucency and GVBD; the latter was done using fluorescence live imaging, followed by visualization of the precipitated GV (5% acetic acid in PBS) and histological analysis of follicles sampled at different time points. The response of follicles obtained from the transgenic *ddx4-EGFP* zebrafish was equal to the one observed in the wild-type AB line, whereby all doses of DHP-induced GVBD in 92 ± 8% of them, while the rate of hCG-stimulation was significantly lower (31 ± 15%). The transgene encodes DDX4 (Vasa) protein, a putative RNA-helicase, which in vitellogenic/postvitellogenic follicles predominantly localizes in the nuclear region. Accordingly, the highest EGFP signal was observed in the GV area, which enabled clear visualization of its migration to the periphery and subsequent dissolution during hormone treatments - in both live imaging and histological samples. As a very small number of follicles ovulated *in vitro*, this process was promoted by gentle pipetting and a combination of DHP, hCG, and PGs (mainly 10 µg/ml $PGF_{2\alpha}$). Although higher ooplasm translucency closely followed nuclear changes, discrepancies were observed with varying conditions. Most notably, final maturation rates in media with different pH were similar; however, follicles incubated in a more alkaline environment (pH 9.0) in some instances reached full ooplasm translucency faster than GVBD, as opposed to ones incubated in pH of 7.5, where the two events were more synchronized. Therefore, *ddx4*:EGFP follicles are a valuable model for parallel tracking the responses of both cytoplasmic and nuclear markers to different maturation conditions, while also allowing further fertilization and/or genetic manipulation of *in vitro* matured oocytes or eggs.

Funding: National Research, Development and Innovation Office of Hungary (NKIFH_K138425).

[P6] Germ cells derived from haploid embryos undergo genome doubling and generate functional gametes in medaka (*Oryzias latipes*)

Takeuchi, M.[1*], Fujimoto, T.[1], and Nishimura, T.[1]
[1]Faculty and Graduate School of Fisheries Science, Hokkaido University, Hakodate, Japan

*Presenting author: takeuchi.moe.w7@elms.hokudai.ac.jp | *Student*

Haploid cells contain a single set of chromosomes and provide the basis for genetic analysis where mutating any recessive genes enables phenotypic characterization. However, in vertebrate, haploids are embryonic lethal and difficult to be maintained *in vitro* because of genomic instability. In mammals, haploid embryonic stem (ES) cells often undergo genome doubling to become diploid and gain genomic stability. Moreover, haploid ES cells can differentiate into germ cells and produce gametes after transplantation into diploids. In fish, haploid cells can recover survival capacity in diploid individuals. However, it remains unknown whether haploid cells in fish undergo genome doubling. In addition, characterization of germ cells derived from haploids and their competence for gametogenesis are not well known. In this study, we aimed to reveal potential for gametogenesis and genome doubling in germ cells derived from medaka haploid embryos.

Medaka haploid embryos were generated by fertilization of eggs with UV irradiated sperm. We found that the medaka haploid embryos with haploid syndrome formed germ cells but died before hatching. To ask whether the haploid germ cells have a potential to undergo gametogenesis, we transplanted EGFP-labeled germ cells from *vasa-EGFP* transgenic haploid embryos into sterile diploid embryos to generate haploid-diploid (1n-2n) germline chimeras. Both male and female 1n-2n chimeras were fertile and produced EGFP-positive diploid progeny by mating with wild-type medaka, indicating that germ cells derived from haploid embryos can differentiate into functional sperm and eggs. Histological analysis of testes from 1n-2n chimeras revealed that all types of germ cells including spermatogonia, spermatocytes and spermatids were present. Moreover, chimeric ovaries also contained oogonia and oocytes (follicles). To ask whether haploid germ cells underwent genome doubling, we sorted EGFP-positive germ cells from 1n-2n chimeric testes and analyzed ploidy of the sorted cells by flow cytometry. The testes of 1n-2n chimeras contained 1n, 2n and 4n germ cells, which was the same ploidy profile of wildtype diploid testes. Altogether, we conclude that haploid germ cells potentially undergo genome doubling possibly before entering meiosis and produce functional sperm and eggs via normal gametogenesis, which are thought to be isogenic. We propose that germ cells with homozygous mutations can be efficiently induced by mutation of a haloid genome and subsequent genome doubling, providing a valuable tool for the study of gene function.

Funding: JST FOREST Program, Grant Number JPMJFR210D (TN).

[P7] Molecular characterization and gonad expression pattern of dead-end (*dnd*) gene in European sea bass (*Dicentrarchus labrax*)

Djellata, A.[1,2], Zapater, C.[1*], Ibañez, S.[1], and Gómez, A.[1]
[1]Fish Reproductive Physiology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castelló, Spain
[2]Aquaculture Research Group, IU-ECOAQUA, University of Las Palmas de Gran Canaria (ULPGC), Telde, Las Palmas, Spain

*Presenting author: cinta.zapater@csic.es

The gene dead end (dnd) is recognized for its critical role in germ cell development and is maternally provided within the germ plasm. It encodes an RNA-binding protein essential for the formation and migration of primordial germ cells, which are pivotal in transferring genetic information to offspring. Characterized across numerous vertebrate lineages, *dnd* has recently been targeted to induce sterility in certain fish species. Despite its significance, there is a lack of information about this gene and its expression pattern in European sea bass (*Dicentrarchus labrax*).

In this study, and departing from different genome predictions, we have determined the complete coding sequence of sea bass dead-end (*sb-dnd*) cDNA, set up a specific qPCR assay and analyzed its expression levels in different sea bass tissue samples. Measurement of *sb-dnd* mRNA levels in adult male and female sea bass tissues revealed a gonad-specific expression pattern. In adult gonadal tissues, *sb-dnd* transcripts were detected in both testes and ovaries throughout the annual reproductive cycle, with notably higher expression in ovaries compared to testes.

In ovarian tissue, expression of *sb-dnd* was consistently high during previtellogenic stages, decreasing as vitellogenesis and maturation progressed, and rising sharply in post-spawning stages, aligning with the proliferation of oogonia and the generation of new oocytes. In testicular tissue, the highest expression of *sb-dnd* was found in spermatogonia-containing and premeiotic stages and decreased during spermatogenesis. Expression of this gene was also analyzed in embryos, newly hatched larvae and gonads of prepubertal animals. In newly hatched larvae, *sb-dnd* expression gradually decreased with larval growth, suggesting a diminishing role as development progresses. In prepubertal animals, gonad *sb-dnd* expression started from the period of gonad sex differentiation, with higher levels in females than in males, similar to the pattern observed in adult animals. The gonad-specific expression and variation during different reproductive stages suggest that *sb-dnd* plays a significant role in germ cell differentiation and development. These findings provide a detailed overview of *sb-dnd* expression in European sea bass and highlight its potential as a target for inducing sterility.

In conclusion, our study enhances the understanding of *sb-dnd* in European sea bass, offering valuable insights into its functional roles and potential applications in aquaculture management, particularly for inducing sterility.

Funding: Spanish MCIN/AEI/10.13039/501100011033 and by ERDF a way of making Europe (PID2021-122929OB-C32). A.D. was supported by Margarita Salas program from ULPGC.

[P8] Cryopreservation of Piracanjuba ovarian tissue in biodegradable capsule

Freitas, T. R.[1], Marques, L. S.[2], Lozano-Torres, K. G.[2], Santos, R. S.[1], Dantas, R. V.[1], França, T. S.[1], Albuquerque, E.[3], Siqueira-Silva, D. H.[3], Zhang, T.[4], Streit Jr., D. P.[1,3*]

[1]AQUAM Research Group, Animal Science Research Program, Federal University of Rio Grande do Sul, Brazil

[2]Oxidative Stress Laboratory, Federal University of Pampa, Brazil

[3]Graduate Program in Animal Reproduction in the Amazon (ReproAmazon) of the Federal Rural University of the Amazon and Federal University of Para

[4]Faculty of Science and Technology, Bournemouth University, Poole, United Kingdom

*Presenting author: danilo.streit@ufrgs.br

Cryopreservation of fish ovarian tissue is an important technique for preserving maternal genetic material. However, this technique uses cryotubes, which are plastic containers with low degradability, generating waste for the environment. The use of biodegradable capsules has proven to be a viable alternative for cryopreservation of fish semen (*Rhandia quelen*), however there are still no studies with ovarian tissue. Therefore, this study aims to evaluate the efficiency of gelatin capsules as a container in the vitrification of ovarian tissue from piracanjuba (*Brycon orbignyanus*), an endangered species. The experiment was carried out in a completely randomized design with fragments of ovarian tissue from three piracanjubas distributed in two treatments (CRIO: vitrified tissue in a cryotube; and CP: vitrified tissue in a capsule). After heating the samples, membrane integrity was evaluated by FDA and propidium iodide (live/dead), mitochondrial activity (MTT), membrane integrity after 24h in *in vitro* maturation medium (0.5 µg/mL FSH and 0.5 µg/mL LH) and in hormone-free culture medium and histomorphometry. There was no significant difference between recipients in membrane integrity (CRIO: 64.04 ± 5.5%; CP: 68.31 ± 10.76%) and mitochondrial activity (CRIO: 8.68 ± 2.66AU/g; CP: 10.29 ± 4.48AU/g). After 24h in cultivation medium (CRIO: 52.30 ± 6.07%; CP: 49.85 ± 8.02%) and maturation medium (CRIO: 49.38 ± 7.37%; CP: 50.52 ± 7.12%), no difference was observed between recipients. No maturation was observed and the perimeter (CRIO: 3.13 ± 1.0mm; CP: 3.44± 0.90mm) and area CRIO: 0.063 ± 0.02mm; CP: 0.61± 0.01mm) of the oocytes did not differ between treatments incubated with hormone. Indicating that vitrified oocytes remain viable after 24 hours, however, it is necessary to improve the *in vitro* maturation protocol. The results of this work demonstrate that the cryotube can be replaced by the gelatin capsule, reducing waste in the environment. In addition to being made from biodegradable material, the capsules are low-cost and easily found on the market.

Funding: Danilo Pedro Streit Jr. (305387/2022-7), Thaiza Rodrigues de Freitas (141423/2021-8) and Francielli Weber Santos Cibin (306037/2023-8) are research fellows from the National Council for Scientific and Technological Development (CNPq-Brazil). Additionally, we extend our appreciation to Energetica Barra Grande S/A (BAESA) for their main financial support [grant number P&D ANEEL 03936-1410/2021].

Session CRYO

[P9] Relationship between seminal plasma composition and sperm quality parameters of the catfish *Pseudoplatystoma reticulatum*

Ninhaus-Silveira, A.[1*], Sanchez, M. P.[1], Borges, L. P.[1, 2], Lobato, S. I. R.[1, 2], Carneiro-Leite, L.[1], Kasai, R. Y. D.[3], Ribeiro, C. S.[4], and Verissimo-Silveira, V.[1]
[1]Laboratory of Neotropical Ichthyology (LINEO), Department of Biology and Zootechnics (DBZ), São Paulo State University "Júlio de Mesquita Filho" - UNESP, Ilha Solteira, SP, Brazil
[2]Post Graduation in Biological Sciences: Zoology, UNESP, Botucatu, SP, Brazil
[3]PIRAÍ Fish-farming, Terenos, MS, Brazil
[4]Laboratory of Studies in Animal Physiology (LEFISA), DBZ, UNESP, Ilha Solteira, SP, Brazil

*Presenting author: alexandre.ninhaus@unesp.br

Sperm quality is a fundamental parameter for the effective reproduction of fish in captivity and the development of reproductive techniques, such as semen cryopreservation. This study aimed to determine the composition of the seminal plasma of *Pseudoplatystoma reticulatum* and analyze the relationships between plasma components and sperm characteristics. Nine males were induced to spermiation with carp pituitary extract in the reproductive period of the species (November and December/2019). Semen characteristics were evaluated: subjective sperm motility, motility, duration, released sperm volume, sperm concentration, pH, osmolality, seminal plasma composition, including levels of calcium, chloride, sodium, magnesium, potassium, glucose, fructosamine, triglycerides, and total protein. To determine the relationship between seminal plasma components and sperm motility parameters, a principal component analysis (PCA) was performed. The seminal plasma of P. reticulatum is composed mainly of the Na^{2+} ion and organic components such as protein and glucose. Through PCA, it was observed that sperm motility had a strong positive correlation with motility time, sperm concentration, and total protein and a negative correlation with osmolality and fructosamine.

Funding: Coordination for the Improvement of Higher Education Personnel/CAPES (88887.302629/2018-00); São Paulo State Research Support Foundation/FAPESP (2020/15020-0).

[P10] Preliminary effects of broodstock diet composition on freezing tolerances of intratesticular spermatozoa of Atlantic salmon (*Salmo salar*)

Figueroa Villalobos, E.[1*], Merino, O.[2], Amorim Pereira, W.[3], Pérez-Atehortúa, M.[1], Niedmann, P.[1], Avila, S.[1], Risopatrón, J.[2], Farías, J.[4], Pinheiro R.[3], Sandoval-Vargas, L.[1], Valdebenito, I.[1], and Villasante, A.[5]

[1]Núcleo de Investigación en Producción Alimentaria, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile
[2]Centro de Excelencia de Biotecnología de la Reproducción (BIOREN-CEBIOR), Facultad de Medicina, Universidad de La Frontera, Temuco, Chile
[3]Laboratório de Biomoléculas Microbianas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Brasil
[4]Departamento de Ingeniería, Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Temuco, Chile
[5]Laboratorio de Biotecnología, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile

*Presenting author: efigueroa@uct.cl

The development of new diets for fish is a constant challenge in aquaculture. The substitution of fishmeal and oils with derivatives of animal origin, terrestrial plants, and microalgae is of interest for improving the quality and tolerance of sperm to cryopreservation in fish reproduction. In this study, the effect of different diets on the tolerance of Atlantic salmon sperm to cryopreservation was evaluated. Four experimental groups were established: Diet Group 1: contained exclusively marine-origin protein and lipid ingredients, Diet Group 2: substituted 65% of the proteins and 51% of the lipids with terrestrial animal and plant sources, Diet Groups 3 and 4: consisted of commercial diets. The broodstock males were fed for a period of six months. Sperm quality evaluation was performed on sexually mature males; samples were extracted by testicular maceration and stored at 4 °C. Subsequently, they were cryopreserved in Cortland medium supplemented with 1.3 M DMSO and 0.3 M glucose in 0.5 ml straws (IMV), frozen in liquid nitrogen vapors, and thawed in a thermostatically controlled bath. The concentration was adjusted to 2x10^9 sperm/mL for further analysis. The results showed that the sperm from Diet Groups 1 and 2 had a higher percentage of Viability and plasma membrane integrity (PMI: 77±6.4% / 80±9.1%), Mitochondrial membrane potential (MMP: 53±8.4% / 50±7.5%), motility rate (MR: 68±8.7% / 63±7.5%) and lower production of cytoplasmic superoxide anion (O_2^-: 33±5.7% / 37±6.4%) and DNA fragmentation (DNA_{frag}: 2.7±0.8% / 3.4±0.6%) compared to Diet Groups 3 and 4 (PMI: 67±4.6% / 62±4.6%; MMP: 48±6.7% / 34±5.5%; RM: 57±7.4% / 51±7.1%; O_2^-: 51±7.4% / 47±6.5%; DNA_{frag}: 5.1±0.7% / 3.7±0.4%; $p<0.05$). Regarding the activity of 8-oxoguanine glycosylase (OGG1) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), an increase was observed in Diet Groups 3 and 4, although without significant differences. In conclusion, our preliminary results suggest that diet composition affects the quality and tolerance of intratesticular sperm to cryopreservation. However, further studies are needed to assess their fertilizing capacity.

Funding: Fund for the Promotion of Scientific and Technological Development of ANID, FONDECYT/INICIACIÓN (Grant Number 11230690) Chile.

[P11] Trying to improve cryopreservation protocols in elasmobranch sperm using new cryoprotectants and biodegradable vials

Magny, A.[1], Franca, T. S.[1*], García-Salinas, P.[1-2], Pérez, L.[1], Asturiano, J. F.[1], and Gallego, V.[1]

[1]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Spain

[2]Research Department, Fundación Oceanogràfic de la Comunitat Valenciana, Valencia, Spain

*Presenting author: thalesfranca@gmail.com

Elasmobranchs, which include sharks, rays, and skates, are among the most threatened groups of vertebrates on Earth. The primary threats to their survival are overfishing and habitat destruction caused by climate change. To address this, both *in situ* conservation efforts and *ex situ* conservation programs are essential. Implementing assisted reproductive technologies, though not widely developed for elasmobranchs, is a crucial aspect of these *ex situ* measures. This study aimed to improve the current cryopreservation protocols in elasmobranch sperm using new cryoprotectants and, in addition, assessing the use of biodegradable vials.

The model species used on these trials was the small-spotted catshark (*Scyliorhinus canicula*), that can be regularly found as part of the fishery by-catch and later kept in a RAS (Recirculating Aquaculture System) system. The sperm was collected the day of the experiment, and it was cryopreserved in 2 mL cryotubes (CT) following the protocol developed by García-Salinas et al. (2021). In this trial, we also used a series of different combinations of cryoprotectants (methanol, MET; dimethyl-sulfoxide, DMSO; glycerol, GLY; and ethylene-glycol, ETG) and two final concentrations (10 and 20%) according to 6 different protocols: MD-10: 5% MET + 5% DMSO; MD-20: 10% MET + 10% DMSO; GLY-10: 10% GLY; GLY-20: 20% GLY; ETG-10: 10% ETG; and ETG-20: 20% ETG. Sperm samples were observed before and after cryopreservation using a microscope, and videos of the sample were then analyzed manually, and the percentage of motile and non-motile spermatozoa were recorded. In general, results showed that post-thawed samples showed significantly lower motility values than the fresh samples (which had around 60-65%). The best results were obtained by the MD-10 combination, with 25% of motile spermatozoa post-thawing, followed by the MD-20 combination, with post-thawing motility values of 15-20%. On the other hand, the other cryoprotectants (GLY and ETG) did not produce good results, probably due to the high toxicity of these cryoprotectants. This was more pronounced in the use of GLY, when sperm sample just before cryopreservation process (after incubation time), showed a dramatic decrease from 60 to 30% for a period of 15 min. Finally, with the best protocol from the first trial (MD-10), new 1-mL biodegradable capsules (BC) were tested as an alternative plastic vial. First attempts showed that traditional vials (i.e. cryotubes) showed better results (30-35% of sperm post-thawing motility) than BC vials (20-25%). However, further studies using these biodegradable containers should address the topic of freezing rates, which could improve the final motility of gametes.

Funding: This work has been funded by the Spanish Ministry of Science and Innovation (ELASMOREP Project; PID2022-138847-I00). VG has a contract (Beatriz Galindo, BG22/00024) funded by the Ministerio de Universidades (Spain).

[P12] Short-term storage in elasmobranch sperm using different pHs and temperatures

Magny, A.[1], Franca, T. S.[1*], García-Salinas, P.[1-2], Pérez, L.[1], Asturiano, J. F.[1], and Gallego, V.[1]

[1]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Spain

[2]Research Department, Fundación Oceanogràfic de la Comunitat Valenciana, Valencia, Spain

*Presenting author: thalesfranca@gmail.com

Elasmobranchs are one of the most threatened vertebrate groups on the planet. The main reasons for that are overfishing and their habitat destruction due to climate change. The increase of the temperature and the acidification of the oceans will have negative effects on the reproduction of many aquatic species. That is why *in situ* conservation tasks, and *ex situ* conservation programs can be used to improve the situation of these endangered species. The application of assisted reproductive control methods, which have sadly not been extensively developed in elasmobranchs, is a necessary component of these *ex situ* measures, even though their efficacy has not been sufficiently demonstrated. The general goal of this study was to compare different methods of short-term storage in elasmobranch spermatozoa.The model species used on these trials was the small-spotted catshark (*Scyliorhinus canicula*), that can be regularly found as part of the fishery by-catch and later kept in a RAS (Recirculating Aquaculture System) system. The sperm was collected the day of the experiment, and it was diluted 1:10 in Eppendorfs of 2 ml following the protocol developed by García-Salinas et al. (2021). For the trials, two different pHs (6.5 and 7.8) and two different temperatures (4 and 20 °C) were tested. Samples were observed using a microscope (10x magnification lens) and videos of each sample were then analyzed manually, and the percentage of motile (displacing, rotating, or actively beating flagella) and non-motile spermatozoa were recorded. Sperm motility in control group (day 0) was 70-75%, and it was checked over 1 month. In general, sperm samples storage at 4 °C (independently of pH incubated), showed higher motility than samples incubated at 20 °C in every checking point. Samples incubated at 20 °C showed statistical differences respect to the control group at 1-day storage (motility of 50-55%, independently of the pH). In addition, sperm motility was below 10% at 3-day storage at this incubating temperature. On the other hand, sperm samples storage at 4 °C did not showed statistical differences respect to the control group (day 0) until 3-day storage time, showing values between 55-60% at both pHs. From that point, the motility of sperm samples storage at 4 °C decreased to 45–50% after 7 days, and below 40% after 14 days (without significant differences between pHs). From 2 weeks of storage, sperm motility samples showed a progressive reduction until day 30, with significantly higher motility values of samples incubated a pH 6.5. Summing up, this study reveals that the best method to store spermatozoa for a short-term period is keeping the samples at 4 °C at pH of 6.5, being possible to reach 40% of motility up to 14 days of storage.

Funding: This work has been funded by the Spanish Ministry of Science and Innovation (ELASMOREP Project; PID2022-138847-I00). VG has a contract (Beatriz Galindo, BG22/00024) funded by the Ministerio de Universidades (Spain).

[P13] Conservation of the brazilian ichitiofauna: National germoplasm bank

Streit Jr., D. P.[1,2], Rodrigues, R. B.[1], Rodrigues, T. F.[1], Tiersch, T.[3]
[1]Aquam Research Group, Animal Science Research Program, Federal University of Rio Grande do Sul, Brazil
[2]Veterinary Science Research Program, Federal University of Rio Grande do Sul, Brazil
[3]Aquatic Germplasm Genetic Resource Center – Louisiana State University (AGGRC/LSU), US

*Presenting author: danilo.streit@ufrgs.br

In Brazil, as well as in the United States, there is a collection of biological material under the custody of their respective governments, CENARGEN and USAD, respectively. However, the reserve composition of samples from the collections of these countries has a significant difference. While in the United States 5.8% of freshwater species present in the country have cryopreserved samples, in Brazil only 0.27% of freshwater ichthyofauna are cryopreserved. Some points can be considered, such as: the richness of species diversity, in Brazil (2,592) four times greater than in the USA (795) and in the American collection there are cryopreserved species that are not native to its ichthyofauna (tilapia, zebrafish and carp). Another important fact to emphasize concerns the composition of a genetic bank that includes the main species of the Brazilian ichthyofauna. A consensus among researchers suggests choosing considered umbrellas- species, which is related to their importance in the eco-biological flow of the ecosystems in which they are inserted. In this initial analysis of the CENARGEN collection, the lack of information regarding the genetic diversity of the donor breeders of the saved samples is a fact. When we analyze the information from the species with the highest number of cryopreserved sperm samples (*Colossoma marcropomum*) all are from captive animals. It is worth remembering that this species is a protagonist in the Amazon system, in addition to being the main South American native species produced in Brazil. However, in a study conducted in 2018 with *C. macropomum*, the low genetic variability of the main juvenile production laboratories of the species in Brazil was observed. Genetic introgression is one of the main concerns related to the loss of genetic variability in wild populations and has been studied for more than two decades in salmon, and should be strongly considered for *C. macropomum*. Another point considered fragile for the CENARGEN samples is the security of the seminal cryopreservation protocols that were used for the formation of this bank. Of the seven species that have cryopreserved sperm at CENARGEN, none has a defined protocol, considered efficient, safe and widely validated. The only South American native fish species that has these pre-requirements is the *Rhamdia quelen*, of which, by the way, there are no samples conserved in CENARGEN. Finally, we understand that Brazil must make a significant effort in order to increase the representativeness of the main species of the Brazilian ichthyofauna, characterize the population genetic profile of the donors and seek improvement in the investigation of cryopreservation protocols for these species.

[P14] The ploidy level of different Acipenseridae species does not influence optimal spermatozoa concentration at cryopreservation: the evidence from sperm subpopulation study

Sotnikov, A.[1], Rodina, M.[1], Gela, D.[1], Boryshpolets, S.[1], Kholodnyy, V.[1], Krasilnikova, A.[1], Lebeda, I.[2], Siddique, M. A. M.[1], Vazačová, M.[1], Pšenička M.[1], and Dzyuba, B.[1*]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic

[2]Biology Centre of the Czech Academy of Sciences, Institute of Hydrobiology, Na Sádkách 702/7, 370 05, České Budějovice, Czech Republic

*Presenting author: bdzyuba@frov.jcu.cz

All sturgeon species are endangered, and their conservation is of utmost importance. Sperm cryopreservation is an effective method for the long-term storage of their genetic material. However, cryopreservation causes cell damage, necessitating optimal protocols modified explicitly for each species. One of the critical parameters for developing a cryopreservation protocol that can be optimized is spermatozoa concentration and its connection with the so-called "cell packing effect". While different sturgeon species respond similarly to external factors, variations in spermatozoa size associated with their ploidy levels may complicate the standardization of cryopreservation protocols. This study investigated the impact of sperm concentration on cryopreservation outcomes in sturgeon species with 2n or 4n ploidy. To provide a wide range of sperm concentrations for study, sperm from each species was concentrated before cryopreservation and diluted with seminal plasma from the same individuals in various proportions to simulate different natural concentrations. After that, the samples were frozen in 0.5 ml straws using a uniform protocol (passive cooling above the liquid nitrogen) and cryoprotective media dilution. The results indicate that ploidy level and spermatozoa size do not significantly differ in the optimal concentration For Siberian sturgeon (*Acipenser baerii*, ploidy 4n, optimal concentration = 0.8×10^9 spz mL^{-1}), Starry sturgeon (*Acipenser stellatus*, ploidy 2n, optimal concentration = 1.1×10^9 spz mL^{-1}), and Beluga (*Huso huso*, ploidy 2n, optimal concentration = 1.0×10^9 spz mL^{-1}), the optimal concentrations were similar to previously reported data for sterlet (*Acipenser ruthenus*). Principal component analysis of motility parameters, obtained using CASA, showed no significant differences between sperm subpopulation structure in experimental groups cryopreserved at different cell concentrations for Siberian sturgeon and beluga. However, Starry sturgeon samples cryopreserved at higher spermatozoa concentrations showed differences from other groups. Motility parameters changed after cryopreservation similarly for all tested species and various concentrations, suggesting that spermatozoa concentration during cryopreservation is a crucial factor for cell post-thaw survival.

Funding: Czech Science Foundation (No. GACR 22-14069S), Ministry of Education, Youth, and Sports of the Czech Republic (project "CENAKVA ", LM2018099).

[P15] Optimizing extenders for short-term storage of sterlet (*Acipenser ruthenus*) sperm in hatchery condition

Shazada, N. E.[1], Zhang, S.[1], Siddique, M. A. M.[1], Cheng, Y.[1], Alavi, S. M. H.[2], Rodina, M.[1], and Linhart, O.[1]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany 38925, Czech Republic

[2]Department of Animal Biology, School of Biology, College of Science, University of Tehran, Tehran 14176-14411, Iran

*Presenting author: nshazadas@jcu.cz | *Student*

Considering endangered fish, storage of sterlet (*Acipenser ruthenus*) gamete is crucial, and investigating the most suitable extender for short-term storage of sperm is necessary. Therefore, the present study aimed to optimize suitable extenders for short-term storage of sterlet spermatozoa and determine the effects of diluted and undiluted stored sperm on fertilization and hatching success in hatchery conditions. The sperm of five mature males with good spermatozoa motility (~80%) were diluted with 20 different extenders having various Na^+ and K^+ concentrations and osmolality. Spermatozoa motility performances were assessed using a CASA system within 144 h storage at 0-2 °C. Undiluted sperm was used as a control. Seminal plasma Na^+, K^+, Ca^{2+} and Cl^- concentrations were 17.56±10.12, 2.48±1.14, 0.11±0.06, and 6.20±3.42 mmol/L with osmolality of 35.60±20.70 mOsm/kg. A two-way ANOVA showed significant effects of storage time, extender, and their interaction on spermatozoa motility, curvilinear velocity (VCL), and straight-line velocity (VSL) ($p<0.001$). The model was decomposed into one-way ANOVA to study the effects of storage time and extenders. Excluding 1 h post-storage, spermatozoa motility, VCL, and VSL were lower in the non-diluted sperm than those diluted with E18-E20 at 24 h post-storage ($p<0.05$). The osmolality of E18-E20 was 46, 55, and 62 mOsmol/kg, respectively, and contained 1 mM KCl. Increasing the extender KCl concentrations to >3 mM resulted in lower spermatozoa motility performance. When the effects of storage time were studied, spermatozoa motility, VCL, and VSL in non-diluted sperm and those diluted with E18-E20 were decreased at 24-48 h and 72-96 h post-storage, respectively. This study shows that extenders with higher osmolality (39-62 mOsm/kg) and lower K^+ (1 mmol/L) are the most suitable for storing sterlet sperm for a short period. After 168 h post storage, sperm diluted with extender E18–E20 were fertilized freshly ovulated eggs and achieved 30.67-31.66% fertilization and 27.95-30.50% hatching success. The malformation rate of the hatching larvae was very minimal (less than 1%) with 168 h post-storage sperm. An extender containing 16-24 mM NaCl, 1 mM KCl, 0.1 mM CaCl2 10 mM Tris, pH 8.0 with osmolality higher than that of seminal plasma should be used to improve and retain the functionality of sterlet sperm during short-term storage.

Funding: Ministry of Education, Youth and Sport of the Czech Republic (LM2023038), Czech Science Foundation (23-06426S), National Agency for Agriculture Research (QK21010141).

Session ENV

[P16] Short-term direct exposure to silver nanoparticles and silver ions impairs sperm motility in Pacific oysters (*Magallana gigas*)

Fernández-García, F.[1*], Carvalhais, A.[2], Marques, A.[2], Oliveira, I. B.[3], Guilherme, S.[2], Oliveira, H.[2], Oliveira, C. C. V.[4], Cabrita, E.[4], Asturiano, J. F.[1], Pacheco, M.[2], and Mieiro, C.[2]

[1]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Spain
[2]CESAM and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal
[3]CIIMAR, University of Porto, 4450-208 Matosinhos, Portugal
[4]CCMAR, University of Algarve, Campus Gambelas, 8005-139 Faro, Portugal

*Presenting author: ffergar@doctor.upv.es | *Student*

The current study aimed to assess the reproductive toxicity of silver nanoparticles (Ag NP) at environmental (0.25 μg/L) and supra-environmental levels (25 and 250 μg/L) on the Pacific oyster (*Magallana gigas*) sperm quality. The significant interest in Ag NPs stems from their wide applications in various fields, such as antimicrobial coatings, healthcare products, and electronics. However, alongside their beneficial uses, concerns about potential toxic effects have also been reported. For the sperm quality analysis, we evaluated sperm motility, mitochondrial function, and oxidative stress. To discern whether the toxicity of Ag stemmed from the nanoparticles (NP) or their dissociation into silver ions (Ag^+), equivalent concentrations of Ag^+ were tested. We found no dose-dependent responses for Ag NP or Ag^+, as both diminished sperm motility similarly without impacting mitochondrial function or causing membrane damage. We suggest that the toxicity of Ag NP primarily arises from their adherence to the sperm membrane or by inhibiting membrane ion channels. The presence of silver in the marine environment may impact oysters' reproduction due to their spermiotoxicity.

Funding: J.F. Asturiano [MCIN - NextGenerationEU-PRTR-C17.I1 and GVA-THINKINAZUL/2021/012) to SEASPERM]; A. Carvalhais (SFRH/2020/05105/BD); I. Oliveira (CEECIND/01368/2018); C Mieiro (DL57/2016).

[P17] Unveiling the mechanisms involved in glyphosate male reproductive toxicity

Lombó, M.[1,2*], Giommi, C.[1], Maradonna, F.[1], Pinto, G.[3], Serpico, S.[3], Illiano, A.[3], Habibi, H.[4], Amoresano, A.[3], Carnevali, O.[1]

[1]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy
[2]Department of Molecular Biology, Universidad de León, Campus de Vegazana, 24071, León, Spain
[3]Department of Chemical Sciences, University of Naples Federico II, Via Cinthia 26, 80126 Naples, Italy
[4]Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada

*Presenting author: mloma@unileon.es

Glyphosate (GLY) is the primary active component of glyphosate-based herbicides, which account for one-third of all herbicides used in European cropping systems and non-agricultural areas. Consequently, the widespread use of GLY has led to the contamination of surface waters, compromising water quality, and endangering aquatic ecosystems. Despite these issues, European agencies do not classify GLY as an endocrine-disrupting chemical, and its regulation has been an intricate topic due to significant economic implications. In this study, we exposed male zebrafish for 21 days through the diet to GLY doses within the limits established by the European Food Safety Authority: 0.5 mg/kg bw/day (acceptable daily intake), 5 mg/kg bw/day, and 50 mg/kg bw/day (no observed adverse effect level) to assess its toxicity on male steroidogenesis and spermatogenesis. After the treatments, targeted testicular metabolomics revealed increased levels of cAMP, AMP, and GMP in males exposed to the highest dose. Additionally, the evaluation of gene expression in the testes of these males showed an overexpression of *amh*, *star* and *cyp11a1.* Since the last two encode proteins mediating the initial and rate-limiting step in steroidogenesis, we therefore measure the levels of steroid hormones by LC/MS-MS. Results indicated increased levels of pregnenolone, progesterone, dehydroepiandrosterone, and 17β-estradiol (E2) in the testes of males exposed to the highest GLY dose. These data suggest that the increase in testicular cAMP triggered by exposure to 50 mg/kg bw/day may underlie the higher expression of *amh* in Sertoli cells and of steroidogenic genes in Leydig cells, thus leading to elevated levels of certain sex steroid hormones, such as E2. Both the increase of *amh* expression and E2 levels in male zebrafish disrupt spermatogenesis by causing excessive proliferation of spermatogonia, as we have also observed in males exposed to 50 mg/kg bw/day GLY after carrying out the testicular morphometric analysis. This, along with a significant increase in germ cell apoptosis, resulted in a dramatic impaired reproduction of these males exposed to the highest GLY dose. Such findings highlight the endocrine-disrupting activity of GLY at testicular level and underscore the urgent need to review the safety levels of GLY established by regulatory agencies.

Funding: This work received funding from the Fondo Ateneo 2022 to FM and OC.

[P18] Water quality and impacts of Barra Bonita reservoir water in zebrafish spermatogenesis

Moraes, A. C. N.[1] and Nóbrega, R. H.[1]
[1]Reproductive and Molecular Biology Group, Department of Structural and Functional Biology, Institute of Bioscience, São Paulo State University (UNESP), Botucatu, Brazil

*Presenting author: rafael.nobrega@unesp.br

Barra Bonita reservoir is in the Tietê River Basin - São Paulo state – Brazil. This reservoir is utilized for several activities such as fish production, irrigation, navigation, tourism, and recreation, besides hydroelectricity production. Human activities in the watershed produce considerable impact such as the discharge of untreated wastewater, the high suspended material contributions, and fertilizers from the sugar cane plantations. Therefore, the increase in pollutants and the lack of inspection of the emission of chemical compounds in natural environments, has increased the risks and mortality of natural species and, consequently, human contamination. In this work, we will assess water quality as well as water toxicity, and its effects in fish gonads exposed in vivo. We will attempt to identify possible altered biological pathways, as well as possible biomarkers for environmental contamination. Additionally, we hope to contribute to the elucidation of new mechanisms of action of environmental contaminants and in the awareness and inspection of the dumping of industrial and domestic waste in natural environments. Water samples were collected (22°31′39.0″S 48°30′11.0″W) on the wet period of 2024 (February) and water parameters and phytoplankton counting and identification were evaluated. Sexually mature male zebrafish (4–5 months-old) were exposed to water from Barra Bonita reservoir for 48h, 7d and 14 days (n = 5 for each period evaluated). Handling and experimentation were performed according to Ethical Principles in Animal Research (Protocol n. 3549051223-CEUA). For the quantification of the relative number of spermatozoa, twenty-five different histological fields were captured at 100x objective lens and analyzed by IMAGEJ Software. Total RNA from testes was extracted using TRIzol™ (Invitrogen, Carlsbad, CA, United States), and quantity and purity were checked with a NanoDrop™ One Spectrophotometer (Thermo Scientific, Madison, WI, United States). cDNA synthesis was performed and, qPCR reactions were conducted using SYBR-Green Universal Master Mix. The relative mRNA levels of stereoidogenesis enzymes (*cyp17a1*, *star*), growth factors (*igf3*, *amh*), spermatogonial markers (*dazl*, *pou5f3*), meiotic marker (*sycp3l*), apoptosis marker (*noxa*), receptors (*ar*, *ers1*, *ers2b*) were measured in the different treatments and normalized by β-actin levels. We observed that regarding water quality, chlorophyll-a (58 ug/L), total nitrogen (4.99 mg/L), total phosphorus (0.2 mg/L L) were above the maximum limit for Class 2 waters. Trophic state index was hypereutrophic. Cyanobacteria cell counting was 223.140 cells/mL (CONAMA Limit 357/05: 50,000 cells/mL). Phytoplankton density (org./mL) was 29.578 (75%) of Microcystis aeruginosa. In addition, our results showed water from Barra Bonita reservoir can impair gene expression (*amh*, *igf3*, *star*, *ers1b*) as well as decreased spermatozoa cells in adult male zebrafish after 14 days. This approach allowed us to evaluate the subacute effects of combine contaminants present in Barra Bonita water reservoir in male zebrafish.

Funding: This research is supported by São Paulo Research Foundation (FAPESP), Brazil – (2023/01319-2 granted to ACNM; 2021/06742-5 granted to RHN).

[P19] Drugs, neurotransmitters, and fish sperm function

Boryshpolets S.[1*], Kholodnyy, V.[1], Sotnikov, A.[1], Dyčka, F.[2], Sterba, J.[2], and Fedorova, G.[1]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Czech Republic

[2]University of South Bohemia in České Budějovice, Faculty of Science, Institute of Chemistry and Biochemistry, Czech Republic

*Presenting author: sboryshpolets@frov.jcu.cz

The presence of pharmaceutical active compounds (PAC) in surface waters and their effects on aquatic life at environmentally relevant concentrations have resulted in growing concern and provoked more studies in this field. After exposure to PAC, pathological and behavioural changes were observed in fish and crayfish. PAC interact with neurotransmitter (NT) signalling, which has specific target receptors in neuron cells. These receptors are well conserved across different species, suggesting similar mechanisms in humans and fish. Thus, pharmaceuticals designed for humans could directly affect aquatic organisms and exhibit similar biological effects in the aquatic environment if effective drug concentrations reach the blood plasma of fish. In addition, specific NT receptors were observed in invertebrates, mammalian testis, and spermatozoa, suggesting that neuronal signalling is important not only for brain function but also for reproduction. Moreover, the expression of receptors on spermatozoa may also suggest NTs' direct effect on sperm functioning and performance during reproduction. Our experiments were aimed to estimate the possible effect of PAC and NT on spermatozoa quality at three levels in European perch (*Perca fluviatilis*) as a primary target species: 1) *in situ*, sperm were sampled from fish growing in different localities (a pond with high PAC concentrations and a control pond); 2) *in vivo,* sperm were sampled from fish exposed to different PAC in aquaria; 3) *in vitro*, the direct effect of PAC and NTs present in swimming media, or during sperm preincubation were tested. Despite significant variability in the size of individual perch males (of the same age) between two different localities, we did not observe any significant changes in sperm quality between these two groups. Similarly, we did not observe any significant effect of PAC exposure on sperm motility, volume, and concentration. However, significant changes were observed in NT expression in the testis and sperm of exposed fish. Several NTs, whose receptors have been confirmed by protein analyses, were selected for *in vitro* experiments, and their synaptic concentration was used. Among several tested NTs, dopamine, choline, gamma-aminobutyric acid (GABA), and tyramine demonstrated the strongest effect on spermatozoa motility after 24 h incubation. In some cases, dopamine, choline and GABA positively affected sperm performance. In contrast, tyramine inhibited spermatozoa motility after 30 s preincubation in perch. The observed effect of tyramine and the possible impact of other NT at synaptic concentration suggest their potential interaction with Ca signalling in spermatozoa and require further detailed study.

Funding: Czech Science Foundation [project No. 22-03754S], South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses CENAKVA (LM2023038).

[P20] Multi-omics assessment of gametogenesis in polar cod exposed to crude oil

Fernández-Míguez, M,[1*], Erhart, C.[2], Yadetie, F.[1], Karlsen, O. A.[1], Goksøyr, A.[1], Odei, D. K.[2], Sørensen, L.[3], Hosseinzadeh, M.[4], Porte, C.[4], and Nahrgang, J.[2]
[1]University of Bergen, 5006 Bergen, Norway
[2]UiT - The Arctic University of Norway, N-9037 Tromsø, Norway
[3]SINTEF Ocean, N-7465 Trondheim, Norway
[4]Institute of Environmental Assessment and Water Research (IDAEA), 08034 Barcelona, Spain

*Presenting author: maria.miguez@uib.no

Understanding the impacts of crude oil exposure to critical life stages in fish is crucial to safeguard the reproductive health and sustainability of fish populations. Oil exposure may lead to endocrine disruption and impact gonad maturation. The present study aims to map the molecular targets and pathways affected by crude oil toxicity and link them to the reproductive success of polar cod (*Boreogadus saida*), a key species in the arctic marine ecosystem at risk from oil spills. Following 3 weeks exposure of fish to weathered crude-oil under vitellogenesis, we analyzed the transcriptome (RNA-seq) in the tissues of the pituitarygonad-liver (PGL) axis as well as the proteome (proteomics) and lipidome (lipidomics) in spawned eggs. Accumulation of oil compounds in the brain, gonad and liver was observed, with highest occurrence of naphthalene and cyclic monoaromatic hydrocarbons in all tissues. Polycyclic aromatic hydrocarbons (PAHs) represented the smallest fraction in all tissues. Transcriptomic analysis showed effects on several genes in many cellular processes including aryl hydrocarbon mediated xenobiotic metabolism and stress response pathways in all tissues. Gonad transcriptome showed modulation of genes related to lipid metabolism, which might be related to alterations of lipid composition as suggested by the lipidomics analysis (e.g. reduced triglycerides synthesis). Ultimately, this work will provide important insights on metabolic pathways affected by oil and its effects on egg quality.

Funding: Research Council of Norway (Toxigen project no. 334541).

[P21] Environmental levels of titanium dioxide nanoparticles compromise the gonads of Pacific oyster (*Magallana gigas*): gender-specific effects

Fernández-García, F.[1*], Marques, A.[2], Jerónimo, S.[3], Oliveira, I. B.[4], Carvalhais, A.[2], Pereira, V.[2], Asturiano, J. F.[1], Pacheco, M.[2], and Mieiro, C.[2]

[1]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Spain
[2]CESAM and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal
[3]Agricultural College of Coimbra (ESAC/IPC), 3045-601 Coimbra, Portugal
[4]CIIMAR, University of Porto, 4450-208 Matosinhos, Portugal

*Presenting author: ffergar@doctor.upv.es | *Student*

This study aimed to evaluate the environmental impact of titanium dioxide nanoparticles (TiO_2 NPs) on the gonadal health of Pacific oysters (*Magallana gigas*) as a proxy for reprotoxicity. Oysters' exposure was via water for 3 and 7 days to two environmentally relevant concentrations of TiO_2 NPs (10 and 100 μg/L). Afterwards, sex was determined, and the oxidative stress profile (antioxidants and damage), energy-related parameters [energy available (carbohydrates, lipids, and proteins), energy consumption (ETS)], and cellular energy allocation (CEA) were assessed. Our findings revealed distinct response patterns for exposure duration, concentration, and sex. After 3 days of exposure, and for the 10 μg/L concentration, the males' ETS decreased, demonstrating mitochondrial function impairment, while after 7 days, males faced depletion of the carbohydrate levels and oxidative stress. Oxidative stress also occurred at 100 μg/L. CEA increased at both exposure periods and for 100 μg/L. The antioxidant levels of females decreased after 3 days of exposure to TiO_2 NPs concentrations, but no oxidative stress occurred. Simultaneously, carbohydrates also decreased after 3 days at 100 μg/L concentration, suggesting the mobilization of the energy reserves to keep the metabolic homeostasis, though ETS and CEA were unaltered. These outcomes underscore TiO_2 NPs' potential reprotoxicity to Pacific oysters' males at environmentally relevant concentrations through oxidative stress-mediated mechanisms, setting 10 μg/L as reprotoxic to males. Females indicated susceptibility to 100 μg/L of TiO_2 NP, though was not possible to establish a critical threshold for reprotoxicity. This research provides valuable insights into understanding TiO_2 NPs-induced impacts at environmental concentrations, suggesting gonads as a sensitive indicator of these NP toxicity and highlighting the potentially harmful effects on bivalves' reproduction.

Funding: J.F. Asturiano [MCIN – (NextGenerationEU-PRTR-C17.I1) and GVA-THINKINAZUL/2021/012 to SEASPERM)]; A. Carvalhais (SFRH/2020/05105/BD); I. Oliveira (CEECIND/01368/2018); C Mieiro (DL57/2016).

Session QUAL

[P22] Apoptosis in oocyte aging and its impact on embryonic development in common carp *Cyprinus carpio*

Konar, E. S. M.[1*], Mai, K.[2,3], Brachs, S.[2,3], Waghmare, G. W.[1], Policar, T.[1], and Samarin, A. M.[1]

[1]Research Institute of Fish Culture and Hydrobiology, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodňany, Czech Republic
[2]Department of Endocrinology and Metabolism, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany
[3]DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, Germany

*Presenting author: ekonar@jcu.cz | *Student*

Post-ovulatory aging may disrupt fertilization success and impair the oocyte quality, resulting in poor embryo development. The present investigation sought to unravel the fate of *in vitro* aged oocytes in common carp (*Cyprinus carpio*). Fertilization ability was completely lost after 12 hours of post-stripping (HPS), and subsequently, apoptosis was triggered in the advanced stage of oocyte aging. This included an increase of proapoptotic transcripts (*fas*, *bax*, *cathepsin D*, *caspase 8*, *caspase 9*, and *caspase 3a*), elevated caspase protein amount, and activation of a key apoptotic player known as caspase 3 in aged oocytes. Furthermore, the effects of oocyte aging on the embryonic apoptosis machinery were studied in 5-hour post-fertilized (early blastula) and 24-hour post-fertilized (HPF) embryos produced from fresh and aged oocytes. Expression of apoptotic genes and caspase enzyme activity were stable in early blastula embryos derived from both fresh and aged oocytes. In contrast, the zymogenic and active forms of caspase 3 increased in 24 HPF embryos produced from aged oocytes compared to those produced from fresh oocytes. Thus, apoptosis intensified in 24 HPF embryos derived from aged oocytes without affecting the apoptotic machinery of early blastula embryos. Our findings demonstrate that apoptosis initiated by the Fas system is an important physiological process accompanying oocyte aging in common carp.

Funding: This study was financially supported by the Ministry of Agriculture of the Czech Republic (NAZV QK22020144) and by the Czech Science Foundation (GACR No. 20-01251S).

[P23] Post-thaw storage of European perch sperm affects larval survival

Judycka, S.[1*], Panda, A.[1], Palińska-Żarska, K.[2], Debernardis, R.[1], and Żarski, D.[1]
[1]Institute of Animal Reproduction and Food Research, PAS, Olsztyn, Poland
[2]National Inland Fisheries Research Institute, Olsztyn, Poland

*Presenting author: s.judycka@pan.olsztyn.pl

Fish sperm cryopreservation helps manage the genetic diversity of fish species, facilitates spawning synchronization, and allows for selective breeding. One of the aspects bringing struggle to scientists and aquaculturists is the post-thaw storage of sperm. It has been commonly accepted for years that sperm should be used for fertilization immediately after being thawed. However, recently, it has been suggested that immediate use of cryopreserved sperm for fertilization following thawing is not mandatory to attain successful fertilization in salmonid fish. This suggestion was based on the post-thaw sperm motility and fertilization rate determined at eyed eggs stage and hatching, but there is no information concerning long-term effect of post-thaw sperm storage on subsequent developmental stages, specifically larval performance. Therefore, the aim of this study was to evaluate the phenotypic consequences of post-thaw storage time on larvae of European perch. The 6 males were stripped for milt using a catheter and sperm motility was determined. The sperm cryopreservation was performed using glucose-methanol extender (consisting of a final concentration of 0.3 M glucose, 7.5% methanol and 25 mM KCL at a concentration 3 × 10$_9$/ml spermatozoa) using 0.5 ml plastic straws. Fertilization of eggs was carried out on ribbons collected from 3 females. Each ribbon was divided into four equal portions (about 30 g each) and fertilized with cryopreserved semen coming from two males immediately after thawing (time 0) and 30 min after thawing. During in vitro fertilization sperm:egg ratio of 100,000:1 and 200,000:1 was ensured for time 0 and 30 min, respectively, in order to compensate amount of motile spermatozoa. After hatching standardized zootechnical parameters of larvae were determined: weight (W) and total length (TL) at mouth opening and at weaning, swim bladder inflation efficiency (SBIE, %), mortality and cannibalism. Fresh semen was characterized by 85±6 % of sperm motility, which decrease to 69±5 % immediately after thawing, with further decrease to 27.9±5 % at 30 min after thawing. No significant differences were found in W and TL, SBIE, and cannibalism of larvae between time 0 and 30 minutes after sperm thawing. However, significantly higher mortality of larvae from day 9 post-hatching (DPH) until the end of the experiment (27 DPH) in eggs fertilized with post-thaw stored semen for 30 min was recorded compared to time 0. Our results clearly indicate that survivability of European perch larvae is highly influenced by post-thaw semen storage time used for fertilization. It is important to note that there is no immediate impact on larval mortality at hatching and first days of their life; however, an increase in mortality becomes evident starting at 9 DPH (coinciding with reduction of yolk reservoirs) and persists throughout larval development. Further analysis should include transcriptomics to investigate and uncover the molecular mechanisms underlying this increased larval mortality.

Funding: National Science Center, Poland (NCN) [grant number (UMO-2020/38/E/NZ9/00394)].

[P24] Fatty acid composition influences egg quality and embryo survival in grayling (*Thymallus thymallus*)

Gubanc, K.[1], Levart, A.[1], Kodela, T.[2], and Sušnik Bajec, S.[1*]
[1]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]Fisheries Research Institute of Slovenia, Ljubljana-Šmartno, Slovenia

*Presenting author: simona.susnik@bf.uni-lj.si

European grayling (*Thymallus thymallus*) is an endangered salmonid fish species and its populations have decreased considerably in European waters in recent decades. One of the conservation measures applied to grayling is stocking with hatchery-reared fish. However, rearing grayling in captivity is challenging mainly due to high mortality during embryonic development. One of the most important factors influencing gamete and larval quality is broodstock nutrition that has not been optimized for grayling yet and this might result in low reproductive performance of hatchery-reared grayling. It is believed that optimal fish broodstock feed should be based on biochemical composition of wild origin grayling eggs. We compared biochemical composition of wild and hatchery origin grayling eggs and evaluate fertilization and hatching rate for eggs of each grayling female separately. Correlations between essential fatty acids quantity and ratios with egg parameters were determined. In addition, larval deformations were also recorded. Hatchery origin grayling females were fed commercial trout starter feed, while wild origin females were feeding on food present in their natural environment. The fatty acid composition of grayling eggs varies depending on the origin of the females (hatchery, wild) and most probably depends on the diet of the females. Hatchery origin eggs contain more fatty acids, more MUFAs and PUFAs than wild origin eggs, which could be linked to higher oxidation level and consequently higher embryo mortality in hatchery origin eggs. In addition, hatchery origin grayling eggs are lighter, containing less carotenoids acting as antioxidants, than wild origin eggs, further contributing to potential higher oxidation in hatchery origin eggs. DHA, ARA and EPA, all LC-PUFAs, are considered essential fatty acids and are linked to abnormalities in development, decreased hatching success, and low offspring survival in numerous teleost fishes when present in subnormal levels or ratios. Hatchery origin eggs contain more DHA, while wild origin eggs were characterized with more ARA and EPA. Ratios DHA/EPA and EPA/ARA were higher in hatchery origin eggs compared to wild origin eggs. Higher DHA/EPA ratio in eggs were correlated with higher egg mortality, lower hatching rate and smaller larvae after hatching. Quantity of ARA in grayling eggs is also shown to be important; namely, higher ARA content is correlated with higher hatching rate and larger larvae after hatching. The proportion of deformities was much lower for wild origin than for hatchery origin larvae. Biochemical composition of grayling eggs significantly differs between eggs of hatchery and wild origin, which might have an influence on the embryonic development and hatching success of hatchery origin grayling.

Funding: The work was supported by The Slovenian Research and Innovation Agency (ARIS) (Research core funding No. P4–0220).

[P25] Assessment of some egg quality parameters and chorion ultrastructure characterization in Atlantic salmon (*Salmo salar*)

Pérez-Atehortúa, M.[a, b*], Sandoval-Vargas, L.[b], Risopatrón, J.[c], Farías, J.[d], Figueroa Villalobos, E.[b], and Valdebenito, I.[b]

[a]Doctorado en Ciencias Agropecuarias, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile
[b]Núcleo de Investigación en Producción Alimentaria, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile
[c]Centro de Excelencia de Biotecnología de la Reproducción (BIOREN-CEBIOR), Facultad de Medicina, Universidad de La Frontera, Temuco, Chile
[d]Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Temuco, Chile

*Presenting author: maritza.perez2021@alu.uct.cl | *Student*

Farming salmonid production requires oocytes and embryos of high quality to supply the increasing demand on the market. Nevertheless, both parameters depending on the management of female and gamete. On the other hand, the egg chorion is a crucial structure for embryo protection. However, it is still poor studied in some fish species. The object of the present study was to evaluate some parameters of oocyte quality and embryonic development and to characterize the chorion at the ultrastructural level in oocyte, embryos at different stages and at hatching, through scanning electron microscopy (SEM). To obtain the samples (oocytes and embryos) five mature females (4 years old, 9.75 ± 0.86 kg) *S. salar* were employed. The results show 32 ± 19.2% of oocytes with yolk clots, a hydration percentage of 17 ± 5.4% after three days of incubation and a significant increase in embryo hardness after 24 accumulated thermal units (ATU) and fertilization. It was recorded a mean fertility rate of 77 ± 12.9%. The survival rate was 72 ± 30% and 73 ± 24% for 130 and 318 ATU, respectively. There were high values of symmetrical blastomeres (65 ± 13%). Nevertheless, spine deformities were also high (42 ± 17.7%). There were also several significant correlations between the assessed parameters. In this regard, there was a high-positive correlation between the hydration percentage and blastomeres symmetry (r= 0.89), between oocyte hardness and spine deformities (r= 0.88), between embryo hardness at 130 and 318 ATU (r= 0.97). There were also strong correlations between both symmetry and survival rate at 130 ATU (r= 0.95) and between symmetry and survival rate at 318 ATU (r= 0.96). With regard to ultrastructural analysis, the SEM images reveals that the external side of the chorion of both oocytes and fertilized eggs even at hatching time exhibit a granular pattern. Instead, the internal side is composed of numerous interconnected fibers that form a complex network, which is absent in the hatching area. Both sides show numerous pores. In the external side, the pores are covering by plugs and show a higher size compared with the internal ones. The information generated in this study is valuable to complement the egg biology of *S. salar,* as well as to improve the reproductive management practices in salmonid farms.

Funding: FONDECYT REGULAR 1211246 and FONDECYT INICIACIÓN 11230690.

[P26] Effects of dietary macro-nutrient composition on the reproductive performance and gamete quality of European sea bass broodstock

Sempere, L.[1], Marín, C.[1], Navarro, J. C.[1], Amaral, J.[2], Pohlenz, C.[2], Gómez-Requeni, P.[2], and Felip, A.[1]
[1]Institute of Aquaculture Torre de la Sal (IATS), CSIC, Castellón, Spain
[2]BioMar Group, Brande, Denmark

*Presenting author: afelip@iats.csic.es

Broodstock feeding is crucial for successful reproductive performance and gamete production in fish. It is known that suboptimal feeding conditions seriously disrupt the fecundity and gametogenesis of breeder fish, thus affecting egg and larval size, spawn quantity and frequency as well as sperm concentration and motility, sperm fertilization ability and hatching rate. Therefore, the formulation of optimal diets and the evaluation of their impact on growth and health, as well as on the reproductive capacity of the animals is crucial for the industry. To this aim, two dietary trials were conducted in sea bass broodstock (n = 65-70 fish, from September to April) that were distributed into five 3000-l circular fiberglass tanks (6-7 fish of each sex per tank) at a density of 15 kg m^{-3}. In each trial, fish fed a control diet and two experimental diets that were considered in duplicate. Firstly, we evaluated the ratio of the dietary composition of the essential fatty acids (FA) DHA/EPA/ARA. The control group was designed as fish fed a low dietary ratio, whereas the experimental groups were designed as fish fed medium and high dietary ratios of essential FA. Secondly, we evaluated the dietary supplementation levels of taurine (Tau). The control group was designed as fish fed with low supplemented levels of Tau, whereas the experimental groups were designed as fish fed medium and high supplemented levels of Tau. Fish in both dietary trials were maintained under natural temperature and photoperiod conditions at the IATS facilities (40º5'N, 0º 10'E). Spawn data were recorded during the spawning season (January-March), and reproductive performance of animals was evaluated in each trial according to: i) the number of spawns, ii) the proportion of viable (floating) to non-viable (sinking) eggs, iii) the fatty acid profile of the eggs, iv) the hatching rate, v) the larval survival rate with and without food administration, and vi) the offspring performance including parental contribution as it has significant implications in terms of production goals and fish management. These findings may be of interest for consideration in practical operations for selective breeding programs, as broodstock management is crucial in hatcheries to properly provide good quality fish eggs.

Funding: MCIN PID2019-109548RB-I00 (REPROBOOST), MICIN NextGenerationEU (PRTRC17. I1) and Generalitat Valenciana (THINKINAZUL/2021/042/026).

[P27] Burbot *Lota lota* sperm contains subpopulations with different sensitivity to external Ca^{2+}

Dzyuba, V.*, Sotnikov, A., Mueni, L. M., Kholodnyy, V., Musatova, I., Policar T., and Dzyuba, B.

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic

*Presenting author: vdzyuba@frov.jcu.cz

Burbot, being the only freshwater species of Gadiformes, is characterized by extremely low-temperature spawning conditions, which determine the specific properties of their gametes. The latter is attractive for studying fish gametes concerning the evolution of reproductive systems and ecology. From a reproductive biology perspective, it is not clear why sperm motility activation in burbot is characterized by the high sensitivity to external Ca^{2+} and K^{+} and low sensitivity to external osmolality. Our study was done to elucidate how these properties are related to the essential characteristics of sperm collected at different phases of the spawning season. We performed sperm motility analysis by CASA in sperm samples activated in isotonic to seminal plasma activating medium containing different concentrations of Ca^{2+}. We also measured ATP content in sperm samples before and after motility activation using a luminescence quantification approach. Obtained data were subjected to sperm motility description by principal components calculated based on CASA data. We found that sperm samples are characterized by presence of subpopulations differing in their sensitivity to external Ca^{2+}. The relative amount of spermatozoa in these subpopulations changed according to the phase of the reproductive season. At the start and peak of the spawning season, sperm motility can be activated by 0 mM Ca^{2+} concentration and repeatedly activated by increasing Ca^{2+} concentration to 5 and 10 mM. In this phase of the spawning period, no specific sperm subpopulations were detected. However, at the end of the spawning period, it was possible to activate sperm motility only at Ca^{2+} concentration in activating solutions higher than 1.5 mM. Around 10% of spermatozoa belonged to the fraction sensitive to 1.5 mM Ca^{2+} concentration, while around 70% of spermatozoa could activate their motility at Ca^{2+} concentration not lower than 5 mM. Additionally, sperm motility activation did not lead to suspected sperm ATP level decrease, suggesting the existence of specific to burbot sperm arrangement of bioenergetic pathways. Future research of this phenomenon is required for understanding sperm biology in burbot related to their ecological niche and for the increase of effectiveness of artificial reproduction methods.

Funding: Czech Science Foundation (No. GACR 22-14069S), Ministry of Education, Youth, and Sports of the Czech Republic (project "CENAKVA ", LM2018099).

Session GAM

[P28] Yolk composition, distribution and role in *Mustelus mustelus* a placentotrophic viviparous shark

Chemello, G.[1], Carli, S.[1], Giorgini, E.[1], Trotta, E.[1], Maradonna, F.[1], Carnevali, O.[1], and Gioacchini, G.[1*]
[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

*Presenting author: giorgia.gioacchini@univpm.it

Elasmobranchs have ancestral reproductive systems which offer insights into vertebrate reproductive evolution. The common smooth-hound *Mustelus mustelus*, is a viviparous placentatrophy organism, characterized by widespread distribution worldwide. Placentatrophy is a reproductive strategy which occurs only in viviparous sharks and their gestation can be divided in 3 phases on the basis of embryo nutrition. During the first months, embryos feed on yolk contained in the yolk-sac. Once the yolk is consumed, embryos feed on milk produced by uterus (histotroph). Finally, embryos feed on maternal nutrients absorbed via a placenta-like connection with the maternal uterus. In the Mediterranean basin, the smooth-hound shark (*Mustelus mustelus*) is one of the species that are considered vulnerable to human activities. Conservation efforts necessitate a thorough understanding of its reproductive strategy.

The aim of this study is to improve the knowledge and identify macromolecular changes underlying oocyte growth and maturation during oogenesis in the common smooth-hound *Mustelus mustelus*, a shark species classified as Endangered (EN) by IUCN Red List. In addition, this study focused on the uterine production and role of yolk component during gestation.

Ovarian tissue containing oocytes at different stages of maturation and uterus were sampled from females caught in the Central Adriatic Sea (FAO-Geographical Sub-Area 17, GFCM). Collected samples have been analyzed by histology (H&E and PAS staining) and FTIR analyses.

Epigonal organ, oogonia, previtellogenic follicles and vitellogenic follicles were identified and described histologically and subjected for the very first time to spectral analysis by FT-IR in order to characterize the macromolecular changes during oogenesis of both somatic cells (Granulosa cells and theca cells) and germinal cells. Structural analysis of *Mustelus mustelus* oocytes at different stages of development obtained by histology, was coupled with IR spectral characterization highlighting for the first time the macromolecular changes of zona pellucida, ooplasm, germinal vesicle and yolk globules underlying follicle growth by both structural and macromolecular point of view. Results evidenced proteins, sugars and lipids concentration and distribution within the oocytes are continuously changing in a highly regulated way during oocyte development; in addition, a peculiar uptake of vitellogenin-like macromolecule and yolk component accumulation within the oocyte was evidenced. Finally, for the first time it was evidence that the milk produced by the uterus contains yolk components directly produced by the epithelial cells only during the histotrophic phase of the gestation.

All these results are of great importance to better understand the significance of yolk role on a viviparous placentotrophic organism.

[P29] The potential role of miR-202 in reproductive phenotype of zebrafish (*Danio rerio*)

Abbasi, M.[1*], Janati-Idrissi, S.[1], Nguyen, T.[1], Thermes, V.[1], Babiak, I.[2], and Bobe, J.[1]
[1]INRAE, LPGP UR1037, Fish Physiology and Genomics, F-35000 Rennes, France
[2]Nord University, Faculty of Biosciences and Aquaculture, 8049 Bodø, Norway

ABSTRACT

*Presenting author: marzieh.abbasi@inrae.fr

MicroRNAs are a group of small non coding RNAs (18-22 nucleotides) that are involved in a wide variety of biological processes. In the medaka (*Oryzias latipes*), miR-202 is targeting directly or indirectly the hippo pathway to regulate female reproductive success. It has been reported that miR-202 is the predominant mature form in female gonads and mature oocytes in zebrafish, which suggests an important role in regulating reproduction. The aim of the present study was to understand the regulatory role of miR-202 on male and female zebrafish (*Danio rerio*) reproductive processes. The total number of eggs, number of viable eggs per clutch, fertilization success, and survival success were carefully monitored in wild-type and mutant (knock out of the mir-202 gene) females mated with wild-type males. Our observations showed a decrease in fertilization success, survival and number of viable eggs per clutch in mutant group compared to wild-type fish. In contrast, the total number of eggs did not significantly change. We then quantified the expression of 33 genes of the Hippo pathway in the ovary of both wild-type and mutant fish using quantitative RT-PCR. Significant differences were observed in the expression of 22 genes between wild-type and mutant zebrafish ($P < 0.05$). Together, our data suggest that miR-202 plays a role in zebrafish female reproduction in associations with Hippo pathway-dependent regulations. Further analyses are needed to further characterize underlying molecular mechanisms.

Keywords: MicroRNAs, miR-202, Reproductive phenotype, Hippo pathway, Gene expression, Danio rerio

Funding: PAUSE program and Agence National de la Recherche (ANR) (ANR-21-CE20-0023 MicroHippo) funding, France.

[P30] Intersex gonads: searching for germ cell molecular markers

Paris, A., Ortiz-Zarragoitia, M., and Diaz de Cerio, O.*
CBET+ Research Group, Dept. Zoology & Animal Cell Biology; Faculty of Science & Technology and Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country. Areatza Hiribidea 47, 48620 Plentzia, Bizkaia, Spain

*Lead presenter: oihane.diazdecerio@ehu.eus

Intersex testes characterized by the presence of oocytes within testicular tissue (ovotestes) have been observed in up to 83% of male thicklip grey mullet in the Gernika estuary (Basque Country). In contrast, males from nearby estuaries exhibit normal testicular development, revealing the presence of feminizing contaminants in the area of Gernika. Mullets are considered gonochoristic and maintain separate sexes throughout their lifespan. Genes, hormones, and environmental factors among others influence se differentiation in teleost. This study aims to assess the transcription levels of early germ cell development genes (*FIGLA, LHX8, BMP15*, and *Nanos3*) in thicklip grey mullet (*Chelon labrosus*) to identify early molecular biomarkers of intersex testis. Transcription levels of selected genes were quantified using qPCR in testicular, intersex (low and high severity), and ovary tissues at various developmental stages (previtellogenic, cortical alveoli, and advanced vitellogenic stages). The results were compared against previous RNAseq studies.

qPCR analysis revealed that *FIGLA, LHX8* and *BMP15*, as an oocyte specific genes, were expressed principally in early-stage ovaries and some intersex samples, but not in testes. Additionally, for *Nanos3*, as previous studies predict, its expression was only detected in ovaries showing a high transcription level in previtellogenic stage and decreasing significantly in cortical alveoli and advanced vitellogenic stages. The qPCR results confirmed RNAseq differentially expressed genes. These findings provide knowledge of the role of these genes in mullet sex differentiation and ovary maturation processes. However, in-depth investigations are required to reveal the expression patern and role of these genes involved in mullet ovotestes development and in particular germ cells formation.

Funding: Basque Gov. (IT1743-22), MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

[P31] Identification and the potential role of secreted novel AID/APOBEC-like deaminase 1 (SNAD1) in carp reproductive system

Majewska, A. M.[1*], Dietrich, M. A.[1], Budźko, L.[2], Adamek, M.[3], Figlerowicz, M.[2], and Ciereszko, A.[1]

[1]Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland
[2]Department of Molecular and Systems Biology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
[3]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Germany

*Presenting author: a.majewska@pan.olsztyn.pl

The primary function of fish seminal plasma is to establish an optimal environment for the storage of spermatozoa. The storage conditions are designed to preserve sperm fertilizing capability and motility, as well as to maintain metabolic activity, viability and ensure energy resources for sperm activation.

Using high-throughput LC-MS/MS-based proteomic analysis of fish seminal plasma and the updated *Cyprinus carpio* genome annotation, we detected hundreds of less abundant proteins, in addition to major proteins, including four variants of Secreted Novel AID/APOBEC-like Deaminase 1 (SNAD1), a unique protein characterized by the presence of a signal peptide among all of intracellular classical Activation-induced cytidine deaminase/apolipoprotein B mRNA editing catalytic polypeptide-like deaminases (AID/APOBECs, AADs). This group of proteins is zinc-dependent cytidine deaminases that catalyse the deamination of bases in nucleic acids, resulting in a cytidine to uridine transition and are the central part of antibody diversity and antiviral defense. To date, there is no available knowledge on SNADs including protein characterization, biochemical characteristics and catalytic activity. This study aimed to investigate the expression of the SNAD1 gene in carp tissues (testis and spermatic duct) acclimated to different temperatures and bacterial infection. We found no regulation during temperature adaptation in the testis and spermatic duct; however, SNAD1 was downregulated in the testis during *Aeromonas salmonicida* infection. Moreover, our *in silico* analysis provides strong evidence of the universal presence of SNAD1 proteins/transcripts in fish, in which expression commences after hatching and is highest in anatomical organs linked to the immune system. To date, in several publications, raw results regarding SNAD1 expression changes have been available but were not included in the general discussion due to "uncharacterized" status of sequences. Recently, we identified homology between these sequences and SNAD1, which allowed us to interpret obtained results and speculate about SNAD1 possible functions. We found that SNAD1 expression is highly modulated during immune responses. Several studies have suggested potential roles for this protein in innate immunity, enhancement of innate immunity under cold conditions, stress responses, adaptive immunity in fish and interactions with the intestinal microbiota. Moreover we noted association of SNAD1 with environmental pollution, and sex-based expression differences, with females showing higher levels. Uncovering the biological roles of SNADs represents an exciting new area of research, particularly regarding the role of DNA and/or RNA editing in fish reproductive biology.

Funding: National Science Centre, Poland (grant number 2021/43/B/NZ9/02869), Deutsche Forschungsgemeinschaft (DFG project number 426513195).

[P32] Cold seawater pre-treatments effects on induction of early sexual maturation and sperm production in European eel

Ferrão, L.[1], Morini, M.[1], González-Lopéz, W. A.[1], Gallego, V.[1], Felip, A.[2], Pérez L.[1], and Asturiano, J. F.[1*]

[1]Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Valencia, Spain

[2]Grupo de Fisiología de la Reproducción de Peces, Instituto de Acuicultura Torre de la Sal, CSIC, Castellón, Spain

*Presenting author: jfastu@dca.upv.es

To induce sexual maturation in captive eels, hormonal treatments are commonly employed, although various pre-treatment methods have been explored to enhance hormonal responsiveness. This study assesses the impact of pre-treating male eels with low-temperature (10 °C) seawater for different durations (2 and 4 weeks) on reproductive outcomes. Biometric parameters including eye, gonadosomatic and hepatosomatic indexes were measured in both control (without thermal seawater pre-treatment) and pre-treated fish. Blood and testis samples were collected for sex steroid and histological analyses, respectively. Cold seawater pre-treatment facilitated early sexual maturation, stimulating steroid production, biometric changes and the onset of spermatogonia differentiation. Eels pre-treated for 4 weeks exhibited significantly higher gonadosomatic index and higher levels of T, 11-KT and E2 compared to those in other groups. Conversely, those pre-treated eels for 2 weeks had increased progestin (17,20ß-dihydroxy-4-pregnen-3-one) levels compared to the control group. In the testis of both pre-treated eel groups, there was an increase in the proportion of spermatogonia type B cells, a differentiation process that was not observed in the control group. Once finished the thermal pre-treatments, eels received a standard hormonal treatment with weekly doses of recombinant human chorionic gonadotropin at 20 °C. Pre-treated eels initiated spermiation earlier than the control group. Throughout the hormonal treatment, pre-treated individuals exhibited higher sperm density, motility and kinetic parameters at certain intervals. Thus, this *in vivo* study suggests that cold seawater pre-treatment may enhance sensitivity to standard hormonal maturation treatment. Moreover, the low temperature pre-treatments demonstrated their economic effectiveness in terms of hormone treatment profitability, increasing the production of high-quality sperm in the European eel.

Funding: MCIU (RTI2018-096413-B-I00, EELGONIA; BG22/00024), GVA (GRISOLIAP/2020/ 063). MCIN (EU, NextGenerationEU-PRTR-C17.I1) and GVA (THINKINAZUL/2021/012, -042).

[P33] Heat shock factors in the European eel: gene characterization and expression response to different environmental conditions and to sexual maturation

Ferrão, L., Pérez L., Asturiano, J. F.*, and Morini, M.
Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Valencia, Spain

*Presenting author: jfastu@dca.upv.es

Heat shock factors (*hsf*s) play critical roles as transcription factors, regulating responses to environmental changes and participating in physiological processes such as spermatogenesis and testis maturation. Given the significance of environmental conditions during the European eel reproductive migration, investigating *hsf* expression in this species offers insights into their physiological functions. Initially, we conducted BLAST analyses on vertebrate genomes (including in the European eel) to identify *hsf* sequences. Concurrently, phylogenetic and syntenic analyses were performed to study *hsf* sequence evolution in vertebrates. Our investigation identified five previously described *hsf*s (*hsf1-5*) and a new one, designated as *hsf*6. In the European eel, we identified two *hsf1* paralogs (*hsf1a* and *hsf1b*), likely resulted from the teleost whole-genome duplication event, while other *hsf*s (*hsf2*, *hsf4*, and *hsf5*) existed as single paralogs. Subsequently, we evaluated *hsf* expression levels in various male tissues, revealing a significant expression of all five *hsf* genes in the testis, along with additional detection in the brain, intestine, and gills. Next, we analyzed *hsf* gene expression in testes collected from eels exposed to different temperatures (10 vs. 20 °C) and salinities (freshwater vs. seawater), simulating eel migration conditions. Notably, only *hsf1* genes expression were modulated under varying temperature and salinity conditions, suggesting their role in detecting environmental shifts during male reproductive migration. Finally, we assessed *hsf* gene expression in testes from eels hormonally matured with recombinant human chorionic gonadotropin (hCGrec), inducing sexual maturation. Throughout hCGrec-induced spermatogenesis, all *hsf* genes displayed a decreasing expression profile during testis maturation, with significant differences noted in *hsf1a* and *hsf4*, while *hsf5* exhibited the highest expression levels after 4 weeks of hormonal treatment. Our study highlights the potential importance of *hsf* genes in the European eel gametogenesis, particularly in responding to environmental changes, and might be implicated in the reproductive migration in this teleost species.

Funding: MCIU (RTI2018-096413-B-I00, EELGONIA), GVA (GRISOLIAP/2020/063). MCIN (EU, NextGenerationEU-PRTR-C17.I1) and GVA (THINKINAZUL/2021/012) to SEASPERM.

[P34] Are androgenic steroids the agents of sexual inversion in *Brycon orbignyanus* (Bryconidae) (Valenciennes, 1849)?

Veríssimo-Silveira, R.[1,2*], Quirino, P. P.[1,2], Delgado, M. L. R.[1,2], Gomes-Silva, L.[1,2], Branco, G. S.[3], Moreira, R. G.[3], and Ninhaus-Silveira, A.[1,2]

[1]UNESP/FEIS - Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Ilha Solteira. LINEO - Laboratory of Neotropical Ichthyology

[2]USP - Universidade de São Paulo, Instituto de Biociências, LAMEROA – Laboratório de Metabolismo e Reprodução de Organismos Aquáticos

*Presenting author: rosicleire.verissimo@unesp.br

The piracanjuba, *Brycon orbignyanus*, is a gonochoristic neotropical species of great ecological and commercial interest. When reared in captivity, it differentiates sexually into females, primary males and secondary males that originate from females that undergo sex inversion. The aim of this study was to verify the plasma profile of the androgens testosterone (T) and 11-ketotestosterone (11-KT) throughout the differentiation and sexual inversion of *B. orbignyanus* kept in captivity. To do this, monthly collections (n=15) were carried out from February to December 2018, sampling specimens from two months to one year old. The specimens were euthanized and blood and gonads were collected. The plasma was sent for steroid analysis, quantifying the androgens testosterone (T) and 11-ketotestosterone (11-KT). The gonads were subjected to the usual histology techniques. Among all the animals analyzed, we identified undifferentiated specimens, females at the beginning of differentiation, intersex, males at the beginning of differentiation and females with fully developed ovaries, all considered Immature, as well as males with functional testes considered Spawning Capable. No statistical differences were found in the plasma level to T, while for 11-KT, Spawning Capable males and intersex individuals showed a higher plasma concentration of the androgen compared to the other groups analyzed. Thus, it is suggested that sexual inversion females of *Brycon orbignyanus* is directed by the action of the androgen 11-ketotestosterone.

Funding: FAPESP (Process 2013 / 24527-8 and 2014 / 23379-8), CNPq (Process 302108/2015 -7 and 305673 / 2018-1) and CAPES.

Session AQUA

[P35] Short-term preservation of *Chelon Labrosus* sperm

Martín-Montero, I. E.[1*], Rasines I.[1], Aguado-Giménez, F.[1]
[1]"El Bocal" Marine Aquaculture Plant, Oceanographic Centre of Santander COST-IEO (CSIC), Monte-Corbanera, Santander, 39012 Cantabria, Spain

*Presenting author: nacho.martin@ieo.csic.es

Short-term conservation in aquaculture facilitates the management of artificial reproduction in aquaculture by allowing sperm to be available in a wide window of time to adapt to oocyte stripping and subsequent artificial fertilization. There are several methods and commercial products that optimize this procedure.

The presented study includes the evaluation of 3 short-term variant preservation methods: 1) fresh sperm, 2) sperm - L15 (Leibovitz) at 1:3 and 1:9 dilutions and 3) sperm - HBSS (Hanks' balanced salt solution) at 1:3 and 1:9 dilutions. For this purpose, sperm from 3 *C. labrosus* males was used, fish were housed in 7 m^3 tanks in the aquaculture facilities of the COST of the Spanish Institute of Oceanography-CSIC, in open flow circuit and fed daily *ad libitum* with sea bream pellets supplemented with natural food (mussel and worm) 3 days per week. Experimental dilutions were prepared and evaluations were carried out in a computer assisted sperm analysis system (CASA) from Proiser S.L. at 0, 3, 6, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336, 432, 528, 600 and 696 hours. The samples were kept in a refrigerator (4ºC) during the whole process and were kept on a bed ice while the motility determinations were carried out in the laboratory. In addition, the sperm samples were hand-shaked every 48 h. to avoid excessive precipitation of the sperm cells.

The results showed a more pronounced loss of motility in the sperm samples diluted in L15, with a reduction of 80% at 48 hours and 96 hours with the 1:3 and 1:9 dilutions, respectively. The refrigerated fresh sperm samples showed a progressive loss of motility reaching 80% reduction of the initial motility at 192 hours. On the other hand, samples kept in HBSS at different dilutions maintained 80% of motility until 600 and 696 hours at 1:3 and 1:9 dilutions, respectively. Furthermore, despite the scattering of the data, the samples kept in HBSS at 1:9 dilution showed motility values around the initial one without large oscillations between 264 and 600 hours kept at 4 ºC.

In conclusion, the results obtained show that *C. labrosus* sperm can be preserved in Hanks´balanced salt solution at a 1:9 sperm:preservative dilution, at 4ºC, for up to 25 days without loss of motility. This increases the conservation time by 300% with respect to fresh sperm at 4ºC without preservatives, which is capable of maintaining up to 20% of the initial motility for 192 hours (8 days).

Funding: This research is part of the project "ThinkInAzul" funded by the European Union–NextGenerationEU and by the Government of Cantabria (Spain).

[P36] Salmonids sexing of sperm by flow cytometry

Vidal, R.[1*], Ojeda, G.[2], and Bañares, H.[2]
[1]Laboratory of Genomics, Molecular Ecology and Evolutionary Studies Genomics, Universidad de Santiago de Chile, Santiago, Chile
[2]Biocode Spa, Puerto Montt, Chile

*Presenting author: ruben.vidal@usach.cl

Flow cytometry is a useful technology in the sexed sperm, which measures and analyzes simultaneously, multiple physical characteristics of the cell, as they flow in a stream flow, through a light beam. The measured properties are the size of a particle, relative internal granularity, relative complexity and relative fluorescence intensity. In the animal production sector flow cytometry is a common platform that allows spermatozoa X and Y differentiation by DNA content. Nevertheless, in aquaculture its application has been very little studied. Therefore, in the present work, we show for the first time the application of flow cytometry in salmonids and its usefulness in generating all-male populations. Our results suggest that flow cytometry can be applied for sperm sexing in salmonids.

[P37] Effects of diet and age on the biochemical composition of grayling (*Thymallus thymallus*) eggs and gametes quality

Kodela, T.[1*], Levart, A.[2], Horváth, A.[3], and Sušnik Bajec, S.[2]
[1]Fisheries Research Institute of Slovenia, Ljubljana-Šmartno, Slovenia
[2]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
[3]Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

*Presenting author: tjasa.kodela@gmail.com | *Student*

The European grayling *(Thymallus thymallus),* a species of conservation concern due to declining populations, is a popular target for sport fishing. Supplementation through stocking with hatchery-reared grayling individuals represents a key conservation strategy. Given the established relationship between parental diet and gametes quality in fish, this study investigates the effects of three different commercial diets (low-energy, high-energy and carotenoidrich) on biochemical composition of grayling eggs and gametes quality parameters to improve our knowledge of grayling reproduction in the hatchery. We set up a two year experiment, focusing on first-time spawners in the first year and repeat spawners in the second year. Both age and dietary factors influence quality of grayling gametes. Surprisingly, a greater difference in the fatty acid composition of eggs is associated with the age of the female and not with the diet. Eggs from first-time spawners had higher levels of monounsaturated fatty acids (MUFA) and lower levels of polyunsaturated fatty acid (PUFA) than eggs from repeat spawners. In particular, the DHA/EPA ratio, a recognized indicator of early mortality syndrome, was elevated in first-time spawners. The biochemical profile of the eggs was significantly influenced also by broodstock diet. The carotenoid-rich diet resulted in higher levels of essential fatty acids in the eggs, while the high-energy diet increased long-chain omega-3 PUFAs (LC n-3 PUFA). However, these dietary variations had no significant effect on egg survival. Similar to the findings for egg biochemical composition, sperm concentration and motility were more strongly influenced by age than by broodstock diet. The values for sperm motility and concentration were much higher in repeat spawners than in first-time spawners. Interestingly, we found no major effect of dietary variation on sperm motility. Our study showed that older grayling should be included in reproduction. In addition, the results demonstrated that the commercial feed used for grayling is not optimized for grayling broodstock, which is reflected in the low reproductive parameters of hatchery-reared grayling. The development of broodstock diets that more closely resemble the natural diet of grayling is therefore a key approach to improving reproductive parameters.

Funding: The work was supported by The Slovenian Research and Innovation Agency (ARIS) (Research core funding No. P4–0220).

[P38] Post-thaw survival of cryopreserved larvae of the European flat oyster (*Ostrea edulis*)

Horváth, Á.[1*], Jug-Dujaković, J.[2], Kitanović, N.[1], Balogh, R. E.[1], Marinović, Z.[2], Špelić, I.[3], Barić, O.[3], and Gavrilović, A.[3]

[1]Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Gödöllő, Hungary
[2]Sustainable Aquaculture Systems Inc., Frenchtown, USA
[3]University of Zagreb Faculty of Agriculture, Zagreb, Croatia

*Presenting author: horvath.Akos@uni-mate.hu

Larvae of the European flat oyster (*Ostrea edulis*) were cryopreserved using a controlled-rate freezer in order to investigate their post-thaw survival. Aquaculture of the European flat oyster has suffered from various adverse effects including parasitic diseases that significantly reduced its production. Assisted reproductive technologies are needed to at least partially restore culture of the species. Oyster larvae were diluted in various combinations of extenders (0.4 M sucrose or 0.4 M trehalose), cryoprotectants (ethylene glycol – EG, propylene glycol – PG, dimethyl sulfoxide – DMSO) and additives (polyvinylpyrrolidone – PVP-40). Diluted samples were loaded into either 0.25 or 0.5-ml straws and cryopreserved in a controlled-rate freezer using two cooling profiles. Post-thaw assessment of larval survival was conducted 20 minutes and 24 hours after thawing. Neither the cooling profile, nor the type of extender had a significant effect on post-thaw survival. The type of cryoprotectant, straw volume and the time of assessment (20 min vs. 24 h) on the other hand, had a significant effect on survival rates ($p < 0.001$ for all three). The percentage of surviving larvae was 70–80% at 20 minutes and 40–60% at 24 hours post-thaw. In general, the use of EG and DMSO resulted in higher post-thaw survival percentages than that of PG when 0.25-ml straws were used, however, these were not observed when larvae were frozen in 0.5-ml straws. Further studies are needed to evaluate post-thaw larval survival at later stages and the ability of larvae to form viable spat.

Funding: Projects 2019-2.1.11-TÉT-2020-00247, ÚNKP-23-4-II-MATE-10, K138425, Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences.

[P39] Effect of high rearing temperature on growth and gonad maturation of juvenile male European sea bass (*Dicentrarchus labrax*)

Sarih, S., Zapater, C., Gómez, A., and Felip, A.*
Institute of Aquaculture Torre de la Sal (IATS), CSIC, Castellón, Spain

*Presenting author: afelip@iats.csic.es

The rapid climate change and rising ocean temperatures cause negative impacts on fishes, leading to alterations of metabolic processes, growth and reproduction. The exposure of adult fish to warm temperatures is known to impair reproduction, although the long-term reproductive impact when exposed during early life is not well clarified. This study aimed to evaluate the effects of warm rearing temperatures on growth and gonad maturation in juvenile male European sea bass during their first year of age. Juvenile sea bass (14.0 ± 4.1 g initial body weight) were established in two experimental groups in triplicate: i) natural seawater temperature (control group, CG) (40°08′15″ N; 0°10′12″ E) and ii) 3-4 ºC above the temperature of the control group (temperature group, HTG) for 10 months (July-April). Growth, biometric parameters, testicular growth and development, and circulating plasma levels of follicle-stimulating hormone (Fsh), testosterone (T) and 11-ketotestosterone (11-KT) were analyzed. The elevation of rearing temperature reduced the long-term growth performance of juvenile male European sea bass during their first year of life. The mean weight of males in HTG was approximately 35.8% lower than that of CG. Meanwhile, males in HTG were approximately 12.7% smaller in length than that in CG. The condition factor varied between 1.14 and 1.56 in fish at HTG, while it was around 1.17-1.91 in the CG. The mean condition factor of fish in GC was approximately 3.6% higher than that of HTG. All fish started gametogenesis, but fish in the HTG group showed a delay in the progression towards advanced testicular stages. Accordingly, first significant increase of the GSI was observed after 4 months of experiment in fish of the CG (GSI= 0.47 ± 0.42%) compared to individuals reared in HTG (GSI= 0.05 ± 0.03%). The histological analysis of testis revealed clear differences between CG and HTG, where the percentages of gonadal stage III (mid recrudescence) in the CG reached 29%, the HTG group displayed 0%. In addition, the highest proportion of precociously spermiating males was observed in the CG in February with 78% in comparison to 23% in the HTG. Spermiation lasted longer in the CG (April) in comparison to that of the HTG (March). Also, males in the HTG exhibited a decrease of plasma Fsh, whereas the levels of T and 11-KT remained unchanged in both experimental groups. In conclusion, rising water temperature affects the growth and gonadal development of the European sea bass males during their first year of life by affecting the reproductive axis at multiple levels.

Funding: MICIN, NextGenerationEU (PRTR-C17.I1) and Generalitat Valenciana (THINKINAZUL/2021/042). S.S. supported by a Margarita Salas program from ULPGC.

[P40] Gene expression and phenotypic assessment of egg quality across developmental stages of Atlantic cod

Fernández-Míguez, M.[1,2,5*], Presa, P.[2], Puvanendran, V.[3], Tveiten, H.[4], Hansen, Ø. J.[3], and Pérez, M.[5]

[1]University of Bergen, Bergen, Norway
[2]Universidade de Vigo, Vigo, Spain
[3]Nofima AS, Tromsø, Norway
[4]The Arctic University of Norway (UiT), Tromsø Norway
[5]Instituto Español de Oceanografía, Vigo, Spain

*Presenting author: maria.miguez@uib.no

Egg quality in fishes is commonly determined by fertilization success and cleavage patterns as a phenotypic outcome of underlying regulatory mechanisms. Although these phenotypic estimators of egg quality are useful in farming conditions, these 'good quality' egg batches do not always translate to good larval growth and survival. The identification of genes involved in embryonic development may help finding links between genetic factors of maternal origin and egg quality. Herein, the relative expression of seven stage-specific developmental genes of Atlantic cod were analyzed using quantitative PCR to understand the function of the temporal transcriptome activity during embryogenesis and its relationship with egg quality. Results showed differential gene expression during early development stages and significant transcriptional upregulation from the mid-blastula stage which is a critical point when embryonic genes take the control of development from maternal genes. The comparison of spawning batches showed that the relative gene expression of genes *ccnb2*, *acta*, *tnnt3* and *pvalb1* was significantly higher from the middle of the spawning season where phenotypic quality estimators establish the best egg quality. Moreover, positive significant correlation was observed between quality estimators based on egg morphology and the genetic expression of genes *acta* and *acta1*. This study suggest that the combination of quality estimators, genetics and batch timing could help optimize reproductive protocols for commercial stocks of Atlantic cod.

Funding: This work was supported by the GAIN (Xunta de Galicia) through the IN607B 2018/14 project and ED481A-2017/362 PhD grant. The Cod Breeding Program is funded by the Royal Norwegian Ministry of Trade, Industry and Fisheries.

[P41] Melatonin biosynthesis in the Senegalese sole reproductive axis

Félix, F.[1,2], Oliveira, C.[1], Martín, I.[3], Manchado, M.[4], Cabrita, E.[1,*], and Vera, L. M.[2]
[1]Centre of Marine Sciences (CCMAR), University of Algarve, 8005-139 Faro, Portugal
[2]Physiology Department, Biology Faculty, University of Murcia, 30100 Murcia, Spain
[3]Spanish Oceanography Institute, Santander Oceanographic Centre, 39080 Santander, Spain
[4]Centro IFAPA, Junta de Andalucia, El Toruño, Cádiz, Spain

*Presenting author: ecabrita@ualg.pt

The study of melatonin production in fish gonads is still unexplored and it could contribute to improving the knowledge about its role in reproduction control, especially in species presenting reproductive problems. This experiment aimed to investigate if Senegalese sole (*Solea senegalensis*) testes are an extra-pineal production site of melatonin and, if so, study its seasonal and daily oscillations. For that, wild and F1 broodstocks of Senegalese sole were sampled in the breeding season (BS) and out of the reproductive season (OS), each at mid-light (ML) and mid-dark (MD) times of the day. Fish were euthanized for blood, brain, eye and testis collection. The radioimmunoassay (RIA) was used to quantify the blood plasma melatonin concentration and the expression of genes involved in melatonin biosynthesis (*tph1a*, *tph2*, *hiomt1*, *aanat1a*, *aanat1b*, and *aanat2*) and melatonin receptors (*mel1*, *mel1c* and *mel2*) was evaluated in the target tissues by quantitative real-time PCR (qPCR). In wild males, the concentration of blood plasma melatonin displayed significant day/night differences in both seasons (average ML: 36 ± 22 pg/mL, MD: 108 ± 63 pg/mL), whereas in F1 males the differences were only found in OS (ML: 100 ± 54 pg/mL, MD: 187 ± 88 pg/mL). *Aanat2* gene expression was not detected in the eye, whereas the expression of *mel1c* receptor was not detected in any tissue. Receptors *mel1* and *mel2*, as well as the key enzymes for melatonin biosynthesis *tph1a*, *aanat1a*, *aanat2* and *hiomt1*, were present in the male gonads of Senegalese sole, demonstrating that testes are a peripheral location for melatonin production. The expression of those enzymes and receptors displayed daily and seasonal oscillations in all the tissues in both broodstock origins. However, in all target tissues, the F1 group showed different expression patterns of several genes, compared to the wild animals, which suggests a dysregulation in these fish circadian system: i.e. in wild male gonads, *tph1a* and *aanat2* were upregulated at night in OS, whereas in F1 males no day/night variations were found, irrespective of the season. *Aanat1a* is frequently referred to in the literature as the retinal enzyme, but our study brought the wide tissue distribution of melatonin-synthesis enzymes into discussion, as well as their putative physiological roles in extra-pineal organs, since they were found in Senegalese sole testes. Moreover, altogether, these results proved that Senegalese sole testes are a melatonin production site and, at the same time, highlighted the dysregulation in the hypothalamus-pituitary-gonad (HPG) axis in F1 males.

Funding: EEA grants (PT-INNOVATION-0080), Spanish MINECO (RYC-2017-21835), Portuguese FCT (SFRH/BD/148280/2019, 57/2016/CP1361/CT0007, DOI:10.54499/LA/P/0101/2020).

[P42] Suboptimal culture conditions during zebrafish organogenesis do not impact on the number of *Ddx4*$^{+}$ cells in the genital ridge although cell migration pattern is altered

María Santos-Villangos[1,2*], Valcarce, D. G.[1,2], and Robles, V.[1,2]
[1]Cell Biology Area, Department of Molecular Biology, Universidad de León (ULe), León, Spain
[2]INDEGSAL, Universidad de León (ULe), León, Spain

*Presenting author: msantv03@estudiantes.unileon.es | *Student*

In the field of aquaculture, stress plays a pivotal role as a limiting factor affecting the production of fish species. The early life period represents a window of increased vulnerability to stress. Our starting hypothesis is that stress produced by suboptimal culture conditions (altered photoperiod and temperature) during organogenesis can induce molecular and phenotypic alterations that affect larval behavior and could affect PGC number and migration to the genital ridge in zebrafish (*Danio rerio*) as a model species in aquaculture. The experimental conditions in the stressed group (S^{+}) group included a 24 h darkness photoperiod and constant temperature (T = 34 ˚C) while the control group (C) was standard cultured (14 h light/10 h darkness and T = 27 ± 1 ˚C). The conditions were maintained up to 120 hpf (end of organogenesis in the species). We evaluated daily survival (0-120 hpf), hatching rate at 48 and 72 hpf, malformation rate at 72 and 120 hpf, embryo burst at 24 hpf and larvae swimming pattern at 120 hpf. Regarding gene expression experiments at 120 hpf we focused the evaluation of key genes involved development (*sox2*), stress (*mortalin*), circadian clock (*bmal*, *clocka*), apoptosis (*casp3*, *bip*, *chop*), retinoic acid signaling (*rarga*) and PGC related genes (*ddx4*, *cxcr4b*, *sdf1a*). We also performed at 120 hpf whole mount immunofluorescence (IF) experiments for DDX4 to compare the number and migration of germ line cells in control and malformed larvae from S^{+} group. We showed that suboptimal culture conditions decreased survival rates and increased rate of malformations (mainly related to the absence or inflated swimbladder). The S^{+} conditions reduced the movements of embryos within the chorion at 24 hpf and also increased the levels of immobility in larvae in the experimental group. Regarding the effect of this stress induction protocol on gene expression, we found that after stress induction, some of the studied genes related to stress and circadian clock (*clocka*, *bip* and *mortalin*) were dysregulated. Our whole mount IF experiments revealed similar number of *Ddx4*$^{+}$ cells in C and malformed S^{+} animals, in line with the non-statistically significant differences of *ddx4* gene expression results. However, different non-canonical cell migration patterns of *Ddx4*$^{+}$ cells were registered in the malformed individuals from S^{+} group while stained larvae were analyzed under the stereomicroscope. This observation was supported with the downregulation of *cxcr4b* expression in the samples from sub-optimal cultured larvae. Our results provide evidence that early life stress can induce molecular and phenotypic alterations potentially compromising the development of the gonads.

Funding: This study was supported by grant PID2019-108509RB-I00. DGV was funded by MCIN/ AEI/https://doi.org/10.13039/501100011033, grant IJC2020- 044091-I, and by the European Union Next Generation EU/PRTR.

[P43] Breeders' diet supplementation with Selenium and Zinc or Vitamins C+E modulate different sperm traits in gilthead seabream

Oliveira, C. C. V.[1*], García-Pichel, C.[1], Marrero, C.[1], Fatsini, E.[1], Félix, F.[1], Duarte, D.[1], Parente, P.[1], and Cabrita, E.[1]
[1]CCMAR, University of Algarve, Campus de Gambelas, ed. 7, 8005-139 Faro, Portugal

*Presenting author: ccoliveira@ualg.pt

Gilthead seabream is one of the main produced species in aquaculture, with very standardized reproductive procedures in maternities. However, gamete quality still has space for improvement, namely through the enhancement of breeder´s nutrition and the control of oxidative stress (OS). OS represents one of the principal damages suffered by spermatozoa, that can compromise fertilization success and larval quality. Many are the external factors behind this damage, but nutrition can play an important role in the control of oxidative events by providing adequate compounds that can be transported into the germline. Antioxidants, namely vitamins, minerals and other natural compounds provided in the diet, have a pivotal role in this balance and are especially important during the periods of gametogenesis and spermiation. Thus, in this study, it was pretended to test different antioxidant supplementation in breeders' diet, to improve gilthead seabream sperm traits and to reinforce the sperm antioxidant system. For this purpose, 3 groups of 21 fish were fed with 3 different diets: i) a CONTROL diet by Sparos Lda., ii) a diet supplemented with vitamins C and E (VitC+E), and iii) a diet supplemented with selenium and zinc (Se+Zn). Fish were daily fed on these diets during two consecutive spawning seasons, and sperm samples were collected from each group during the periods fish were spermiating, to assess the following sperm traits in fresh samples: i) spermatozoa motility by CASA; ii) lipid peroxidation by MDA quantification; iii) reactive oxygen species (ROS) and iv) cell apoptosis by flow cytometry. Also, oxidative stress enzymes (Glutathione reductase, GRS, Glutathione peroxidase, GPX and Superoxide dismutase, SOD) and TAS (Total antioxidant status) were measured, respectively in spermatozoa and seminal plasma. The results revealed that each diet modified different sperm traits, in comparison with the control group: VitC+E group had increased sperm velocity at 15 s post activation and reduced lipid peroxidation, while Se+Zn fish showed an increased TAS and reduced SOD activity. No differences between groups were found in ROS, apoptosis status and GRS / GPX activity. These differential modulation of sperm traits suggest that a combined supplementation of these antioxidants could results in improved sperm quality and reinforced antioxidant system in gilthead seabream.

Funding: FCT through project SPERMANTIOX (PTDC/CVT-CVT/4109/2020), CCMAR strategic plan (UIDP/04326/2020, LA/P/0101/2020) and contract 57/2016/CP1361/CT0007 to CCVO.

[P44] The effect of antioxidant supplemented diets and feeding regimes in sperm mRNA abundance of gilthead seabream (*Sparus aurata*)

García-Pichel, C.[1], Cabrita, E.[1], and Fatsini, E.[1*]
[1]Centre of Marine Sciences (CCMAR), University of Algarve, Campus Gambelas, 8005-139, Faro, Portugal

*Presenting author: effernandez@ualg.pt

Broodstock nutrition is considered as one of the main factors that may affect the reproductive success and it is critical to obtain high-quality gametes. There is a tendency to supplement the broodstock diet with polyunsaturated fatty acids (PUFA) making these diets with a high content of fatty acids changing the fluidity of the spermatozoa membrane. This may cause oxidative stress affecting the sperm quality. Antioxidants supplementation in the diet, such as micronutrients like vitamins and minerals can counteract these effects. Vitamin E, C, selenium and zinc have been reported to generate positive results in several fish species. Besides, the feeding period of this supplementation would be also critical depending on the species. All these aspects may reflect on the abundance of certain mRNAs present in sperm acting as quality indicators. The aim of this study was to observe the impact of the dietary antioxidant supplementation in gilthead sea bream (*Sparus aurata*) sperm mRNA abundance according to the feeding regime (short or long feeding period). Adult gilthead sea bream were fed with 3 diets (n = 21 each): A) control, B) vitamins C and E supplemented diet (Vit C+E) and C) selenium and zinc supplemented diet (Se+Zn), for two spawning seasons. In both seasons, males were sampled for sperm collection to evaluate the effect of the short (SP) and long (LP) feeding period on sperm mRNA abundance. RNA was extracted from 100 µl of sperm, cDNA was synthetized, and real-time quantitative PCR (RT-qPCR) was performed to observe the relative expression of the oxidative-stress related genes (*hsp70*, *hsp90* and *oxrs1*), apoptosis (*bax* and *casp3*) and antioxidant activity (*gsr* and *gpx4a*). Significant differences were observed in the expression of *hsp70* between feeding periods in both treatments, Vit C+E and Se+Zn diets. This gene has been associated with anti-apoptotic processes, showing that both supplemented diets enhanced anti-apoptotic cell process at LP. Besides, *oxrs1* was significantly higher expressed in Vit C+E group in comparison with Se+Zn group at LP, showing that at long-term period sperm cells were more protected against oxidative stress in the Vit C+E supplementation. Apoptosis-related genes, *bax* was significantly higher expressed in Vit C+E at SP than Se+Zn group, showing that the Vit C+E decreased the apoptosis in sperm cells. In terms of antioxidant-related genes, *gpx4a* was differentially expressed between Vit C+E and Se+Zn at SP. Both diets presented higher expression of this gene at LP, showing that antioxidant supplementation improved mitochondrial protection of ATP generation against oxidative damage. These results showed that Vit C+E had better results in sperm mRNA abundance and long-term feeding regime was better in protection against oxidative stress and apoptosis.

Funding: FCT- project SPERMANTIOX-PTDC/CVT-CVT/4109/2020; CCMAR - UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020. Contract 2020.04181.CEECIND to E. Fatsini.

[P45] Characterization of sperm subpopulations in two *Leuciscid* species: can the breeding in captivity affect the sperm quality?

Hernández, A.[1], Martínez-Pastor, F.[2], Gil, F.[3], Sousa-Santos, C.[4], Cabrita, E.[1], and Gallego, V.[1,5]

[1]Center of Marine Sciences (CCMAR), University of Algarve (Faro, Portugal)
[2]Institute for Cattle Development and Animal Health (INDEGSAL), University of León (Spain)
[3]Aquário Vasco da Gama (Lisbon, Portugal)
[4]MARE - Marine and Environmental Sciences Centre, ISPA (Lisboa, Portugal)
[5]Grupo de Acuicultura y Biodiversidad, Universitat Politècnica de València (Spain)

*Presenting author: ecabrita@ualg.pt

Populations of freshwater fish species of the Iberian Peninsula have been declining since the mid-20th century, and several types of actions (from *in situ* to *ex situ* measurements) have been applied over the past decades. However, limited knowledge about their reproductive biology makes necessary to investigate different aspects of the reproductive cycle for improving breeding programs, such as the gamete quality. Thus, the main objective of this work was to advance knowledge in the sperm biology of two endemic fish from Portugal rivers, trying to check if the breeding in captivity is a factor able to modulate sperm subpopulations.

Populations of different endangered leuciscid species (*Iberochondrostoma lusitanicum*, IL; and *Achondrostoma occidentale*, AO) were sampled during the spring of 2022 both in captive populations kept at Aquário Vasco da Gama (AVG) and in wild populations (WILD) from different Portuguese rivers. Sperm samples were collected, and sperm motion parameters were assessed by a CASA system (VSL, VAP, STR, LIN, WOB, ALH and BCF). The application of a two-step cluster analysis yielded four sperm subpopulations (SP1, SP2, SP3 and SP4) in both species. SP1 included spermatozoa with high values of VCL, LIN and STR; hence, they were labelled fast and linear spermatozoa. SP2 included spermatozoa with low values of VCL and high values of LIN and STR, then they were labelled as slow linear spermatozoa. SP3 included spermatozoa with high values of VCL and low values of LIN and STR, so they were labelled as fast nonlinear spermatozoa. Finally, SP4 included spermatozoa with low values of VCL, LIN and STR, and they were labelled as slow nonlinear spermatozoa. Looking at the differences between species, results showed that AO presented a higher percentage of fast sperm subpopulation (SP1 and SP3) than IL both in wild and captive animals, while IL showed the highest values of SP3 subpopulation (fast but no linear spermatozoa). Regarding to the origin of fish (wild and captive), and for both species, WILD leuciscids showed a higher values of linear and fast sperm subpopulation (SP1) than captive fish (AVG), which showed a higher percentage of non-linear subpopulations (SP3 and SP4). In this context, and given that fast and linear spermatozoa (SP1) have traditionally been correlated with high fertilization success in many fish species, these results may indicate that breeding in captivity over a long period of time may affect gamete quality, making it necessary to renew the broodstock from time to time to avoid reproductive problems (i.e. loss of gamete quality and cases of inbreeding).

Funding: Funded by MSCA project CRYO-FISH (GA number: 101038049); VG has a contract (Beatriz Galindo, BG22/00024) funded by the Ministerio de Universidades (Spain).

[P46] Relevance of JC-1 for mitochondrial activity measurement in trout rainbow spermatozoa

Labesse, C.[1*], El Kamouh, M.[1], and Labbé, C.[1]
[1]Fish physiology and genomics laboratory, INRAE UR1037, campus of Beaulieu, Rennes, France

*Presenting author: charlene.labesse@inrae.fr

Cryopreservation allows the preservation of genetic heritage and the regeneration of lines particularly in fish farming. However, upon thawing, the spermatozoa suffer several subcellular damages that can have consequences on their fertilizing ability. Mitigation of such damages requires the development of methods to study the quality of cryopreserved spermatozoa. The present work will focus on the mitochondria, whose electron transport chain drives proton transfer from the matrix to the intermembrane space. The created proton gradient will ultimately fuel ATP production via proton reentry in the matrix. Functional mitochondria therefore possess an electrochemical potential whose intensity can be assessed using lipophilic and cationic fluorochromes such as JC-1. In healthy cells with active mitochondria, JC-1 specifically accumulates in the negatively charged mitochondrial matrix to form red fluorescent aggregates (em = 590 nm). In altered mitochondria, inner membrane potential decreases, the interior of the mitochondria is therefore less negative and JC-1 accumulation in the matrix is lessened. JC-1 thus remains in a monomeric form that emits green fluorescence (em = 529 nm). This dual fluorescence is used to express the mitochondrial activity of the cells, where a high red/green fluorescence ratio indicates cells with active mitochondria.

JC-1 is available as a kit and the labeling and assay conditions were optimized for mammalian cultured cells. In this work, we are questioning the conditions of the JC-1 probe use in order to accurately estimate the quality of rainbow trout sperm mitochondria. We carried out various cell labelling tests with JC-1, namely probe concentration, incubation temperature and duration. The fluorescent profiles of the spermatozoa were assessed by flow cytometry and controlled on a fluorescent microscope. We observed that JC-1 concentrations above 0.5 µM induced spermatozoa aggregation, thereby impairing measurement reliability. On the contrary, incubation temperature and duration (20-37 °C and 10-30 min) had little effect on the spermatozoa labeling pattern and we could obtain homogenous cell populations with intense red labelling and stable red/green ratio. The next step was to assess how changes in mitochondrial quality would be conveyed through changes in JC-1 labeling pattern. Various conditions were tested including sperm cold storage (7 days), cryopreservation, and chemical decoupling using the oxidative phosphorylation uncoupler CCCP. The obtained patterns were confusing, either in flow cytometry or microscopy, and the canonical red/green ration did not always faithfully report the changes in cell quality. We discussed that the unique mitochondria behavior in trout spermatozoa may yield a specific response to JC-1 labeling.

Funding: French CRB Anim project ANR-11-INBS-0003.

[P47] Dietary probiotic supplementation from initial feeding to adulthood enhances sperm motility in the model species *Danio rerio*

Sellés-Egea, A.[*1], Martínez-Vázquez, J. M.[2], and Valcarce, D. G.[1,2]
[1]Cell Biology Area, Department of Molecular Biology, Universidad de León (ULe), León, Spain
[2]Instituto Español de Oceanografía, Centro Oceanográfico de Santander (COST-IEO), CSIC, Santander, Spain

*Presenting author: aselle00@estudiantes.unileon.es | *Student*

Lactic acid bacteria (LAB) have been extensively researched for their positive impact on the health of aquatic species. One such strain, *Pediococcus acidilactici* CNCM MA18/5M, is approved for use in aquaculture feed within the European Union under the commercial name Bactocell® (Lallemand). Previous studies have highlighted the beneficial effects of this probiotic on health parameters in various commercially relevant aquaculture species, including rainbow trout, tilapia, or blue shrimp. The present experiment aims to explore the potential beneficial effects of probiotic supplementation throughout the entire life cycle (up to 1 year) using this strain on sperm quality, specifically motility. To validate our hypothesis, we employed zebrafish (*Danio rerio*) as a teleost model species in aquaculture, and the probiotics were bioencapsulated in *Brachionus plicatilis* rotifers (5-20 days post fertilization) and *Artemia franciscana* metanauplii (15 days post fertilization until the end of the experiment). Two experimental groups were created from the same batch, differing only in the feeding program: 1) the control group (CTRL), in which live food was enriched with a commercial product (Easy Dry SELCO®, Inve Aquaculture), and 2) the experimental group (PROBIO), in which live food was enriched with 10^{11} CFU mL^{-1} *Pediococcus acidilactici* MA 15/5M. After 1 year of supplementation, PROBIO fish exhibited similar weight and length compared to CTRL siblings. Sperm samples were collected from 15 males per experimental group following standard protocols and analyzed using computer-assisted sperm analysis (ISAS, Proiser) 15 seconds after sperm activation (> 200 spermatozoa/sample; 3 fields/sample). PROBIO samples showed higher values in total motility (p = 0.0227), progressive motility (p = 0.0339), and the percentage of fast cells (p = 0.0310). In terms of sperm kinetic parameters, PROBIO samples exhibited higher curvilinear velocity (VCL) (p = 0.0399). These results provide evidence of the capacity of bioencapsulated probiotics in live food to improve sperm quality in terms of motility parameters in the model species. The implementation of optimized diet programs incorporating the strain *Pediococcus acidilactici* CNCM MA18/5M throughout the entire life cycle may represent a potentially valuable tool to promote optimal breeders in commercial aquaculture species.

Funding: PROBISOLE project (Fundación Biodiversidad; PLEAMAR2020-FEMP). DGV was funded by MCIN/AEI/doi.org/10.13039/501100011033, grant IJC2020-044091-I, and by the European Union Next Generation EU/PRTR.