CONFERENCE OF EUROPEAN COMPARATIVE ENDOCRINOLOGISTS

Barcelona, 6-10 September 2026

BOOK OF ABSTRACTS

ORGANIZERS

ESCE
European Society for Comparative Endocrinology

Universitat de Barcelona

WELCOME

Dear colleagues and friends,

Welcome to the 32nd Conference of European Comparative Endocrinologists (CECE), held in Barcelona from 6 to 10 September 2026.

The Local Organizing Committee (LOC), under the auspices of the European Society for Comparative Endocrinology (ESCE), has worked with great enthusiasm to offer you a high-quality scientific program, covering a wide range of topics in comparative endocrinology and related disciplines (neurobiology, physiology, biochemistry, ecology, etc.), complemented by an engaging social program.

CECE26 is hosted at the Faculty of Biology of the University of Barcelona (UB), the same venue of the 17th ICCE in 2013. It includes Plenary lectures, Symposia featuring State-of-the-Art presentations and oral communications, poster sessions, as well as Workshops. It brings together researchers and students from across Europe and beyond, promoting interdisciplinary knowledge in the fields of biology and medicine, particularly in the morphological, functional, and evolutionary aspects of comparative endocrinology. Over these five inspiring days, we will explore cutting-edge research, foster collaborations, and share the latest discoveries and innovations in our field.

Social events during the conference include a Welcome Cocktail on Sunday, a social event for young investigators or a visit to the UB's historic building for senior scientists on Monday, excursions to various locations in and around Barcelona on Tuesday, and a Social Banquet on Wednesday.

Barcelona, a vibrant research and innovation hub known for its creativity and scientific spirit, with excellent connections, provides the perfect setting for this meeting, scientific exchange and networking. Beyond the conference, we invite you to experience Barcelona's Mediterranean charm, cultural treasures, and world-renowned cuisine.

We are delighted to welcome you to a stimulating and unforgettable conference, where science, collaboration, and friendship come together.

Warm regards,

Encarni Capilla, Chair
On behalf of the CECE26 LOC

COMMITTEES

LOCAL ORGANIZING COMMITTEE

Encarnación Capilla
Joaquim Gutiérrez
Isabel Navarro
Emilio J. Vélez

INTERNATIONAL SCIENTIFIC COMMITTEE

Jozef Vanden Broeck, Belgium
Encarnación Capilla, Spain
Elisabeth Eppler, Switzerland
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Martina Gáliková, Slovakia
Hamid Habibi, Canada
Angela B. Lange, Canada
Heather G. Marco, South Africa
Rosaria Meccariello, Italy
Luis Yañez-Guerra, UK
Cunming Duan, USA

SPEAKERS

ISABEL BEETS, *Belgium*

Professor, Department of Biology, KU Leuven, Belgium. Research Fellow at the Laboratory of Molecular Biology in the United Kingdom.

OLIANA CARNEVALI, *Italy*

Professor of Developmental Biology and Reproductive Biotechnologies, Department of Life and Environmental Sciences, Polytechnic University of Marche (UNIVPM), Italy.

KENNETH V. HALBERG, *Denmark*

Associate Professor, Section for Cell & Neurobiology, Department of Biology, University of Copenhagen, Denmark.

ANNA JAZWINSKA, *Switzerland*

Professor, Department of Biology, University of Fribourg, Switzerland.

JEFFREY E. PESSIN, *USA*

Judy R. & Alfred A. Rosenberg Professorial Chair in Diabetes Research, Director, Diabetes Research Center, Departments of Medicine and Molecular Pharmacology, Albert Einstein College of Medicine, USA.

PROGRAM AT A GLANCE

CECE26	Sunday Sept 6		Monday Sept 7	
		8:00	Registration desk opens	
		9:00-9:50	Plenary 2 (Aula Magna)	
		10:00-10:30	Symposium 1 (Aula Magna)	Symposium 2 (room M1)
			SoA 1	SoA 2
		10:30-11:00	Coffee Break (Hall)	
		11:00-12:45	Oral (7)	Oral (8)
		12:45-14:15	Lunch (Faculty bar) ESCE council meeting (room A6)	
		14:30-15:00	Symposium 3 (room M1)	Symposium 4 (room M2)
			SoA 3	SoA 4
		15:00-16:45	Oral (7)	Oral (7)
16:00	Registration desk opens	16:45-17:00	Poster pitch oral presentations	
17:15-17:40	**Opening Ceremony** (Aula Magna)	17:00-17:30	Coffee Break (Hall)	
		17:00-18:00	Poster Session 1 (Hall)	
17:40-18:30	Plenary 1 (Aula Magna)			
18:30-20:00	**Welcome Cocktail** (Hall)	18:00-18:30	Workshop (room M1)	Activity ESCE Young Council (room A16)
		18:30-19:00		
		19:00-19:20		
		19:30-20:30 (approx.)	**Social Event (Senior) - Visit to Historic building UB**	**Social Event (Young) -** A15 **Escape room**

Tuesday Sept 8		Wednesday Sept 9		Thursday Sept 10	
Registration desk opens		Registration desk opens			
Plenary 3 (Aula Magna)		Plenary 4 (Aula Magna)		Plenary 5 (Aula Magna)	
Symposium 5 (Aula Magna)	Symposium 6 (room M1)	Symposium 7 (Aula Magna)	Symposium 8 (room M1)	Symposium 11 (Aula Magna)	Symposium 12 (room M1)
SoA 5	SoA 6	SoA 7A	SoA 8	SoA 11	SoA 12
Coffee Break (Hall)		Coffee Break (Hall)		Coffee Break (Hall)	
Oral (8)	Oral (7)	SoA 7B Oral (5)	Oral (8)	Oral (7)	Oral (6)
Lunch (lunch box) Elsevier meeting (sala de juntes) Frontiers meeting (room A6)		Lunch (Faculty bar) ESCE council meeting (room A6)		**ESCE GENERAL ASSEMBLY** (Aula Magna) **Closing Ceremony** (Aula Magna)	
Excursions		Symposium 9 (room M1)	Symposium 10 (room M2)	Lunch (lunch box)	
		SoA 9	SoA 10		
		Oral (7)	Oral (7)		
		Poster pitch oral presentations			
		Coffee Break (Hall)			
		Poster Session 2 (Hall)			
	20:00	**Social Dinner**			

Plenary: 45' + 5'
SoA: 25' + 5'
Oral: 12' + 3'
(#): number of orals in session

SCIENTIFIC PROGRAM

SUNDAY, September 6

16:00	REGISTRATION DESK OPENS	
17:15-17:40	OPENING CEREMONY	*Aula Magna*
17:40-18:30	**PLENARY — PL1** Decoding hepatocyte glucose and fatty acid metabolism at the single cell level Jeffrey E. Pessin | Chair: Encarnación Capilla	*Aula Magna*
18:30-20:00	WELCOME COCKTAIL	*Hall*

MONDAY, September 7

08:00	REGISTRATION DESK OPENS
09:00-09:50	**PLENARY — PL2** A vascular-satellite cell axis drives whole-muscle regeneration in adult zebrafish — *Aula Magna* Anna Jazwinska | Chair: Joaquim Gutiérrez

PARALLEL SESSIONS - MORNING

	ROOM: Aula Magna **Symposium 1** — Invertebrate neurohormones governing physiology and behavior: a symposium honoring the careers of Angela Lange and Ian Orchard (I) *Chairs: Jean-Paul Paluzzi & Meet Zandawala*	**ROOM: M1** **Symposium 2** — Growth and growth factors: A Tribute to Erika M. Plisetskaya *Chairs: Cunming Duan, Munetaka Shimizu & Ya-Xiong Tao*
10:00-10:30	**SoA1** Hormonal control of metamorphosis and reproduction in insects: the case of the desert locust — *Jozef Vanden Broeck*	**SoA2** Beyond calcium regulation: New roles for Stanniocalcins in growth, hypoxic stress, and disease — *Cunming Duan*
10:30-11:00	Coffee Break — *Hall*	
11:00-11:15	**O1** Kinins, pyrokinins and small molecules in mosquito and tick myoactivity and behavior: towards vector-specific pharmacology — *Patricia Pietrantonio*	**O8** Somatostatin regulation of the pancreatic β-cell proliferation — *Adelino Canario*
11:15-11:30	**O2** Neuropeptides controlling diuresis also regulate reproductive processes in female, *Rhodnius prolixus* — *Areej N. Al-Dailami*	**O9** Novel regulators of HIF signaling in zebrafish hypoxia stress model — *Ling Lu*
11:30-11:45	**O3** The integration of multiple modulatory pathways controls juvenile hormone synthesis in insects — *Fernando G. Noriega*	**O10** Gcn2-mediated nutrient sensing orchestrates metabolic homeostasis and hypoxic tolerance in zebrafish — *Chengdong Liu*
11:45-12:00	**O4** Reproductive functions of Diuretic hormone 44 in Drosophila — *Young-Joon Kim*	**O11** The endocrine role of bone in gilthead sea bream *(Sparus aurata)*: systemic effects of BMP7 and SPARC — *Inmaculada Rodríguez*
12:00-12:15	**O5** Blood meal-induced juvenile hormone signaling drives male reproductive maturation in the classical insect model *Rhodnius prolixus* — *Jimena Leyria*	**O12** Role of the venus kinase receptor in the nymphal growth of the desert locust, *Schistocerca gregaria* — *Rong Liu*
12:15-12:30	**O6** Analysis of neuropeptidergic coupling factors controlling multiscale behavioral rhythms in *Drosophila melanogaster* — *Susanne Neupert*	**O13** Cellular and molecular dynamics underlying the transition from hyperplastic to hypertrophic muscle growth in rainbow trout — *Jean-Charles Gabillard*
12:30-12:45	**O7** RYamide signaling controls selective ion reabsorption by the hindgut in the yellow fever mosquito, *Aedes aegypti* — *Jean-Paul Paluzzi*	**O14** Identification of ohnologues transcriptome networks involved in myogenic differentiation of the Atlantic salmon — *Douglas Vernimmen*
12:45-13:00		**O15** Physiological, transcriptional and epigenetic response in rainbow trout *(Oncorhynchus mykiss)* skeletal muscle mediated by chronic stress and infectious pancreatic necrosis virus (IPNV) — *Juan Antonio Valdes*

12:45-14:15	LUNCH — *Faculty Bar* *ESCE council meeting (Room A6)*

Student communication. Eligible for award

MONDAY, September 7

PARALLEL SESSIONS - AFTERNOON

	ROOM: M1 **Symposium 3** — Invertebrate neurohormones governing physiology and behavior: a symposium honoring the careers of Angela Lange and Ian Orchard (II) *Chairs: Jean-Paul Paluzzi & Meet Zandawala*	ROOM: M2 **Symposium 4** — Endocrine control of energy balance and appetite *Chairs: José Miguel Cerdá-Reverter, José Luis Soengas & Suraj Unniappan*
14:30-15:00	**SoA3** Prebilaterian origin of monoaminergic signalling — *Luis Yañez-Guerra*	**SoA4** Endocrine integration of appetite, energy and water balance: lessons from Drosophila — *Martina Gáliková*
15:00-15:15	**O16** Neuropeptide signalling in placozoa — *Gaspar Jekely*	**O23** From intestinal signalling to organismal function: effects of aflatoxin B1 on endocrine–metabolic pathways, feeding and locomotor behaviour in fish — *Ayelén Blanco*
15:15-15:30	**O17** Regulation of simple metamorphosis in insects following the discovery of the Chinmo factor — *Xavier Belles*	**O24** Orforglipron acts as a pharmacological chaperone to correct cell-surface defective human GLP1R variants — *Ya-Xiong Tao*
15:30-15:45	**O18** A mating responsive hormonal program supports female reproductive success during periods of dietary restriction — *Takashi Koyama*	**O25** Ghrelin loss across vertebrate lineages: a consequence of feeding strategies? — *Filipe Castro*
15:45-16:00	**O19** Neuroendocrine disruption in *Daphnia magna*: linking environmental contaminants to behavior, physiology and emerging disease frameworks — *Carlos Barata*	**O26** Cannabinoids modulate the expression level of genes encoding short neuropeptides F (sNPF) and insulin-like peptides (ILPs) precursors in insects — *Szymon Chowański*
16:00-16:15	**O20** The AKH family: invertebrate champions of PTMs — *Gerd Gäde*	**O27** Peripheral FGF21 signalling modulates feed intake and hepatic metabolic pathways in rainbow trout *(Oncorhynchus mykiss)* — *José Luis Soengas*
16:15-16:30	**O21** Peptidergic pathways modulating metabolic homeostasis in Drosophila — *Meet R. Zandawala*	**O28** The role of ITP signaling in the energy metabolism and physiology of *Drosophila melanogaster* — *Sanjay Yadav*
16:30-16:45	**O22** A role for the neuropeptide myosuppressin and its receptors in male reproductive fitness — *Richard Elwyn Isaac*	**O29** Nucleobindin-derived peptides lower lipid levels in hepatocytes — *Suraj Unniappan*

POSTER PITCH ORAL PRESENTATIONS (16:45-17:00)

Room	Time	Code	Author
M1 (Symposia 1, 11)	16:45-16:50	P2	Jimena Leyria
	16:50-16:55	P42	Sajad Shahbazi
	16:55-17:00	P44	Chisato Yatsuda
M2 (Symposium 2)	16:45-16:50	P5	Emilio J. Vélez
	16:50-16:55	P6	Roger Solà
	16:55-17:00	P11	Manel Montblanch

17:00-17:30 Coffee Break *Hall*

POSTER SESSION 1 (Hall) (17:00-18:00)

18:00-19:00 **WORKSHOP** *Room M1*
Regulation of myogenesis: comparative and novel aspects – Jean-Charles Gabillard & Daniel Garcia de la serrana

18:00-19:20 **ACTIVITY ESCE YOUNG COUNCIL** *Room A16*
Topics:
-Grant Writing and Funding Strategies
-Publishing and Journal Selection (including the peer-review process)
-Teaching and Mentoring Skills
-Career Pathways and Postdoctoral Applications
-The Use of AI and Computational Models in Endocrinology

19:30-20:30 *aprox.* SOCIAL EVENT (Senior) - VISIT TO HISTORIC BUILDING UB

19:30-20:30 *aprox.* SOCIAL EVENT (Young) - ESCAPE ROOM *Room A15*

TUESDAY, September 8

08:00	REGISTRATION DESK OPENS
09:00-09:50	**PLENARY — PL3** *Aula Magna* Lighting up neuropeptide signaling in the context-dependent regulation of behavior Isabel Beets | Chair: Jozef Vanden Broeck

PARALLEL SESSIONS - MORNING

	ROOM: Aula Magna **Symposium 5** — Endocrine control of reproduction *Chairs: Oliana Carnevali & Ana Gómez*	**ROOM: M1** **Symposium 6** — Molecular and cellular regulation of stress *Chairs: Juan Miguel Mancera, Lluís Tort & Matt Vijayan*
10:00-10:30	**SoA5** New players, new pathways: emerging mechanisms of gonadotropic regulation of fish spermiogenesis — *François Chauvigne*	**SoA6** Mineralocorticoid receptor activation and stress-related behaviour: insights from single cell sequencing of zebrafish brain — *Mathilakath M. Vijayan*
10:30-11:00	Coffee Break *Hall*	
11:00-11:15	**O30** CRISPR/Cas9 disruption of srd5a2 reveals essential roles of androgen bioactivation in amphibian reproductive maturation and reproductive success — *Diana C. Castañeda-Cortés*	**O38** Mineralocorticoid and glucocorticoid receptor heterodimers drive distinct cortisol-induced stress responses — *Marcel Schaaf*
11:15-11:30	**O31** Differential impairment of ventral and dorsal pituitary gonadotroph function by polyendocrine metabolic ovarian syndrome — *Camilo Fiordelisio-Leal*	**O39** Tissue-specific regulation of cortisol clearance by 11βHSD2 in rainbow trout — *Nicholas Bernier*
11:30-11:45	**O32** Species-specific duplication of gonadal soma-derived factor gene (gsdf) led to sex-biased expression and partial subfunctionalization in European sea bass *(Dicentrarchus labrax)* — *Alessia Mascoli*	**O40** Gut-derived Tachykinins regulate osmotic and nutritional homeostasis in *Drosophila melanogaster* — *Marishia Agard*
11:45-12:00	**O33** Polylactic acid impairs endocrine and reproductive function in mammals: protective effects of *Lemna minor* and comparative insights into sperm physiology — *Maria Zelinda Romano*	**O41** Hypothalamic-pituitary interrenal axis activation in embryonic fishes. Challenging the claim that the axis is hyporesponsive — *Richard Manzon*
12:00-12:15	**O34** Brain-derived neurotrophic factor (Bdnf): a regulator of reproduction in zebrafish? — *Suraj Unniappan*	**O42** Acute stress enhances macrophage responsivity during inflammation in zebrafish — *Erin Faught*
12:15-12:30	**O35** Temporal redeployment of conserved cell-type programmes underlies seasonal spermatogenesis in European sea bass *(Dicentrarchus labrax)* — *Nerea Sanchis*	**O43** Modulation of HPI axis and GH/IGF system through a dietary *Cannabis sativa*-based additive to improve gilthead seabream *(Sparus aurata)* resilience under chronic stress — *Sara Cartan*
12:30-12:45	**O36** Transposable element evolutionary age shapes DNA methylation dynamics during spermatogenesis in European sea bass *(Dicentrarchus labrax)* — *Nuria Paisano*	**O44** When the hive gets high: Cocaine's effects on honeybee biology and society — *Jan Černý*
12:45-13:00	**O37** Evolutionary and functional insights into CB1 signaling in spermatozoa: implications for endocrine control of reproduction — *Marta Lombó*	

12:45-14:15	LUNCH (Lunch box) *Elsevier meeting (Sala de juntas)* *Frontiers meeting (Room A6)*

AFTERNOON

	EXCURSIONS

WEDNESDAY, September 9

08:00	REGISTRATION DESK OPENS
09:00-09:50	**PLENARY — PL4** *Aula Magna* Wiring physiology: neuroendocrine control of organismal homeostasis through organ crosstalk Kenneth V. Halberg | Chair: Angela Lange

PARALLEL SESSIONS - MORNING

	ROOM: Aula Magna Symposium 7 — Thyroid hormone actions in reproduction and development *Chairs: Hamid Habibi & Deborah Power*	**ROOM: M1** **Symposium 8** — Endocrine disruption *Chairs: Juan Ignacio Bertucci & Vance Trudeau*
10:00-10:30	**SoA7A** Evolutionary and functional crosstalks between the thyrotropic and gonadotropic axes — *Sylvie Dufour*	**SoA8** Multi-omic approaches to unravel new mechanisms of action of endocrine disruptors — *Laia Navarro*
10:30-11:00	Coffee Break *Hall*	
11:00-11:15	**SoA7B** Thyroid hormone action in neurodevelopment. A surprisingly early kickoff — *Aurea Orozco Rivas*	**O50** Estrogenic activity assessment of recalcitrant pharmaceuticals found in wastewaters from Ria Formosa using the *Dicentrarchus labrax* estrogen screen (DLES) test — *Federico Clementoni*
11:15-11:30		**O51** Nuclear estrogen receptors of European sea bass *(Dicentrarchus labrax)* as targets of endocrine disrupting compounds: effects on reproduction and risk assessment potential — *Cinta Zapater*
11:30-11:45	**O45** Molecular effects of thyroid hormone and histone methyltransferase Dot1L during *Xenopus tropicalis* metamorphosis — *Louis Emeric*	**O52** Bridging scales in neurotoxicity: multi-omics evaluation of cadmium and ciprofloxacin effects in European seabass *(Dicentrarchus labrax)* — *Mercedes Blázquez*
11:45-12:00	**O46** Thyroid hormone mediation of gonadal differentiation in zebrafish — *Hamid Habibi*	**O53** Chemically-induced molting disruption in arthropods - an issue of increasing environmental concern — *Carlos Barata*
12:00-12:15	**O47** The hypothalamic regulation of the thyroid axis in teleosts: insights from knockout models and novel transgenic tools — *Deodatta Gajbhiye*	**O54** Cellular landscape of tributyltin toxicity using single-cell and spatial transcriptomics in zebrafish embryos — *Liliya Sokalchuk*
12:15-12:30	**O48** Metamorphosis determines immune responses in juvenile *Senegalese sole* — *Deborah Power*	**O55** Chronic exposure to environmentally relevant PFAS mixtures disrupts metabolic activity and tissue organization in human hepatic organoids — *Anna Manzella*
12:30-12:45	**O49** DNA methylation during limb growth at *Xenopus tropicalis* metamorphosis — *Nicolas Buisine*	**O56** Endocrine and molecular disruption in sea urchin larvae *(Paracentrotus lividus)* exposed to citalopram and triclosan under climate change stressors — *Juan Ignacio Bertucci*
12:45-13:00		**O57** Non-EATS modalities in human and environmental endocrine disruption research — *Jorke Kamstra*

12:45-14:15	LUNCH *Faculty Bar* *ESCE council meeting (Room A6)*

WEDNESDAY, September 9

PARALLEL SESSIONS - AFTERNOON

	ROOM: M1 **Symposium 9** — Hormonal control of vertebrate development *Chairs: Yun-Bo Shi & Yong Zhu*	ROOM: M2 **Symposium 10** — Endocrine responses to environmental challenges *Chairs: Elisabeth Eppler & Heather Marco*
14:30-15:00	**SoA9** Induced primordial germ cells (iPGCs) and expanded germline stem cells (eGSCs) for high-efficient fish surrogate reproduction— *Yonghua Sun*	**SoA10** Endocrine responses to environmental challenges: an overview — *Heather Marco*
15:00-15:15	**O58** CRISPR disruption of mineralocorticoid receptor does not affect overall tadpole growth and development and shows aldosterone unnecessary for metamorphosis — *Daniel Buchholz*	**O65** Brown trout hepatocyte spheroids: an alternative 3D platform for assessing endocrine and climate-related stressors — *Tânia Madureira*
15:15-15:30	**O59** Regulation of zebrafish early sex differentiation — *Bon-Chu Chung*	**O66** Embryonic thermal programming modifies thyroid-driven metamorphosis in gilthead sea bream and European sea bass — *Hamed Abdollahpour*
15:30-15:45	**O60** Complementary and additive functions of TRα and TRβ in regulating larval cell death and adult stem cell development during Xenopus intestinal metamorphosis — *Yun-Bo Shi*	**O67** Seasonal dynamics of the ovarian follicular cell transcriptome in European sea bass: insights into steroidogenesis and thermal effects — *Amanda Paz Escorza*
15:45-16:00	**O61** Elucidating the function of the thyroid hormone receptor alpha a (thraa) in zebrafish heart regeneration — *Hui Zhao*	**O68** Photoperiodic sensitivity at puberty in *Oreochromis mossambicus* — *Naghmeh Jafari Pastaki*
16:00-16:15	**O62** Thyroid hormone receptor regulates cellular senescence to affect larval tissue degeneration in *Xenopus tropicalis* — *Wang Shouhong*	**O69** Harnessing postbiotics to enhance resilience to microplastic toxicity: insights from gut microbiome and defensome responses in gilthead seabream — *Sabrin Hmida*
16:15-16:30	**O63** A somatostatin of maternal origin is crucial for early development — *Adelino Canario*	**O70** Integrated physiological and genomic responses of juvenile chub mackerel *(Scomber japonicus)* to acute hypoosmotic stress — *Vuong Nguyen*
16:30-16:45	**O64** Spontaneous maturation of female American eels *(Anguilla rostrata)* in freshwater ponds — *Shixi Chen*	**O71** Endocrine regulation of nutrition, metabolism and fat storage in a changing world — *Elisabeth Eppler*

POSTER PITCH ORAL PRESENTATIONS (16:45-17:00)

Room	Time	Code	Author
M1 (Symposium 5)	16:45-16:50	P16	Leonardo Pablo Madsen
	16:50-16:55	P17	José Carranza
	16:55-17:00	P21	Marina Iglesias
M2 (Symposia 10, 12)	16:45-16:50	P37	Carlos Barata
	16:50-16:55	P40	Sabrin Hmida

17:00-17:30 Coffee Break *Hall*

POSTER SESSION 2 (Hall) (17:00-18:30)

20:00 SOCIAL DINNER – CAN TRAVI NOU

THURSDAY, September 10

09:00-09:50	**PLENARY — PL5** Endocrine system under chemical pressure: cross-species and 3D human models in a One Health framework Oliana Carnevali | Chair: Adelino Canario	*Aula Magna*

PARALLEL SESSIONS - MORNING

	ROOM: Aula Magna **Symposium 11** — Neuropeptides: new and emerging concepts *Chair: Isabel Beets & Luis Yañez-Guerra*	**ROOM: M1** **Symposium 12** — Endocrinology and chronobiology *Chairs: Nicholas S. Foulkes & Jacob A. Smith*
10:00-10:30	**SoA11** Multifactorial control of fish reproduction by the secretogranin system — *Vance Trudeau*	**SoA12** The zebrafish pineal gland reclaims the spotlight — *Yoav Gothilf*
10:30-11:00	Coffee Break *Hall*	
11:00-11:15	**O72** Transcriptomic and neuropeptidomic insights into nervous system regulation of shell biomineralization in the Mediterranean mussel *(Mytilus galloprovincialis)* — *Zhi Li*	**O79** Evolution of light-regulated cell signalling pathways in vertebrates — *Nicholas Foulkes*
11:15-11:30	**O73** Functional diversification of GLWamide-GPCR system in the cnidarian *Nematostella vectensis* — *Cesar Adrian Garcia*	**O80** A model to study conserved food-reward circuits: ghrelin and dopamine during food anticipatory activity in goldfish — *Lisbeth Herrera-Castillo*
11:30-11:45	**O74** Neuropeptides and their potential roles in scleractinian corals — *Ching-Fong Chang*	**O81** Effects of free temperature choice on clock genes, neurogenesis and brain monoaminergic function in Atlantic salmon parr — *Mauro Chivite-Alcalde*
11:45-12:00	**O75** Evidence of family B1 GPCR genes in Cnidaria rewrites their evolutionary history — *João Cardoso*	**O82** Circadian regulation of extracellular vesicles — *Yiqun Wang*
12:00-12:15	**O76** Insulin signalling and ecdysis in the German cockroach — *José Luis Maestro*	**O83** Sex and day/night dependent regulation of muscle and liver proteome — *Tamara Madzi*
12:15-12:30	**O77** Insect neuropeptides influence the electrophysiological properties of mammalian excitable cells — *Pawel Marciniak*	**O84** Behavioural effects of dopamine depletion induced by CRISPR targeting of tyrosine hydroxylase in zebrafish — *José Miguel Cerdá-Reverter*
12:30-12:45	**O78** The mystery of Myoglianin: a key regulator of development in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) — *Solmaz Ghanbari*	

12:45-13:45	ESCE GENERAL ASSEMBLY	*Aula Magna*
13:45-14:15	CLOSING CEREMONY	*Aula Magna*
14:15-15:00	LUNCH (Lunch box)	

POSTERS

POSTERS

Code	Author	Title
1. Invertebrate neurohormones governing physiology and behavior: a symposium honoring the careers of Angela Lange and Ian Orchard		
P1	Yang Zou	Deorphanisation and evolutionary analysis of monoamine GPCRs in the sea urchin *Mespilia globulus*
P2	Jimena Leyria	Evidence for juvenile hormone regulation of cellular and humoral immunity during vitellogenesis in the classical insect model *Rhodnius prolixus*
2. Growth and growth factors: A Tribute to Erika M. Plisetskaya		
P3	Munetaka Shimizu	An *in vivo* effect of recombinant salmonid insulin-like growth factor binding protein-1a
P4	Munetaka Shimizu	Effect of recombinant rainbow trout insulin-like growth factor binding protein-1a on cultured trout muscle cells
P5	Emilio J. Vélez	Endosomal microautophagy (eMI): an unknown player in the regulation of growth and metabolism?
P6	Roger Solà	Insights into cellular signalling cascade elicited by taurine in cultured myotubes from European sea bass *(Dicentrarchus labrax)*
P7	Roger Solà	Transcriptomic insights into two gilthead sea bream *(Sparus aurata)* genetic lines fed with organic diets
P8	Maricela Luna	Inhibition of growth hormone receptor (GHR) activation by pegvisomant exacerbates neuroinflammation and motor deficits following neonatal hypoxia
P9	Ana Cantero-Labrador	Establishment and characterization of two monoclonal bone-derived cell lines from gilthead sea bream *(Sparus aurata)* as *in vitro* models for bone biology research
P10	Manel Montblanch	GH/IGF expression in liver and muscle of European sea bass fingerlings under dietary and exercise treatments
P11	Manel Montblanch	Autocrine mRNA and miRNA effects of IGF-I and IGF-II treatments in IGF of cultured muscle satellite cells in European sea bass *(Dicentrarchus labrax)*
4. Endocrine control of energy balance and appetite		
P12	Koji Murashita	Divergent roles of Cck receptors in digestion, reproduction, lipid metabolism, and growth in medaka
P13	José Luis Soengas	Effects of peripheral cannabinoid receptor 1 blockade on hepatic metabolism in rainbow trout
P14	Esther Isorna	Ghrelin induces while leptin decreases metabolic rate in goldfish
P15	Ayelén Blanco	PCB-126 impairs intracellular and metabolic signalling in rainbow trout intestinal cells
5. Endocrine control of reproduction		
P16	Leonardo Pablo Madsen	Development of a comprehensive proteome database of sperm and seminal extracellular vesicles in a marine fish: a resource for comparative endocrinology and ecotoxicology research
P17	José Carranza	Molecular basis of the action of corticosteroids on oogenesis in the teleost fish medaka
P18	Hirofumi Ohga	Endocrine roles of leptin in gonadotropin regulation and puberty onset in chub mackerel
P19	Brianna Raven	New regulator of reproduction? Secretoneurin localization uncovers strong association with reproductive hormones in brain and pituitary of *Silurana tropicalis*
P20	Cinta Zapater	Development of a specific AMH ELISA for minimally invasive sex determination in loggerhead sea turtle *(Caretta caretta)*
P21	Marina Iglesias	Spatial transcriptomics profiling in European sea bass *(Dicentrarchus labrax)* in maturing testis
P22	Ana Gómez	Involvement of the transcriptional coactivator Ncoa7 in early gametogenesis and puberty of European sea bass *(Dicentrarchus labrax)*
6. Molecular and cellular regulation of stress		
P23	Mauro Chivite	Modulation of juvenile rainbow trout endocrine stress response under cumulative stressors: implications for fish welfare
P24	Irene Lozano	Sexual dimorphism in the epigenetic and transcriptional regulation of gonadal stress responses in fish
P25	Juan Miguel Mancera	Endocrine regulation of stress axis in the teleost fish gilthead seabream *(Sparus aurata)* exposed to high stocking density and dietary nanoplastics
P26	Juan Miguel Mancera	Modulation of stocking density stress response in European seabass *(Dicentrarchus labrax)* through dietary phytogenic supplementation
7. Thyroid hormone actions in reproduction and development		
P27	Itay Oz	Coordinated thyroid axis dynamics describes metamorphosis in the flathead grey mullet *(Mugil cephalus)*

Code	Author	Title
8. Endocrine disruption		
P28	Paolo Cocci	Hepatic miRNome and endocrine-related molecular responses induced by environmentally exposed microplastics in gilthead seabream *(Sparus aurata)*
P29	Emma Moragrega-Knol	Comparative proteomics to investigate neuroendocrine-related molecular responses in *Daphnia magna*
P30	Liliya Sokalchuk	Decoding the molecular fingerprints of organophosphate flame retardants with transcriptomics
P31	Brian Jonathan Young	Divergent modes of action converge on shared adverse outcomes: a multi-level analysis of BPA, PFOS, and TBT
9. Hormonal control of vertebrate development		
P32	Diana C. Castañeda-Cortés	Revisiting the role of prolactin in amphibian metamorphosis
P33	Yong Zhu	Role of metalloproteinases in folliculogenesis in zebrafish
P34	Xiaojian Lai	Spontaneous precocious vitellogenesis of female *Anguilla rostrata* in captive seawater cage
P35	Jing Huang	Bioluminescence of the large yellow croaker: involvement of xanthophore's fluorescence in physiological luminescent color tuning
10. Endocrine responses to environmental challenges		
P36	Isabel Forner	Modulation of fish carboxylesterases by early-life exposure to emerging contaminants
P37	Carlos Barata	Environmental dopaminergic neurotoxicity induces Parkinsonism like phenotypes in *Daphnia magna*
P38	Camila Faria	Combined effects of microplastics and temperature on stress and metabolic responses in juvenile Pacu *(Piaractus mesopotamicus)*
P39	Oksana Raabe	Adipose tissue as an endocrine organ: recent insights into the anatomy and phylogeny of fat storage and regulation
P40	Sabrin Hmida	Sentinels of the Conero: The Wild "Mosciolo" of Portonovo as a place-based education model for climate change awareness in schools
11. Neuropeptides: new and emerging concepts		
P41	Karolina Walkowiak Nowicka	Changes in the expression level of the gene encoding short Neuropeptide F in the beetle *Tenebrio molitor* in response to environmental stress
P42	Sajad Shahbazi	Computational reconstruction and prioritization of neuropeptide receptor candidates in *Tenebrio molitor*
P43	Inga Schirmer	Inside the fly mind: A strategy for spatial multiomics brain imaging to map neurochemical landscapes in *Drosophila melanogaster* by Imaging Mass Spectrometry
P44	Chisato Yatsuda	Long-term effects of intracerebroventricular administration of GnIH on energy homeostasis in male Japanese quail
12. Endocrinology and chronobiology		
P45	Lisbeth Herrera-Castillo	Chronodisrupted feeding alters the responsiveness to ghrelin
13. Miscellaneous		
P46	Mariana Teles	Impact of combined exposure to nanoplastics and high temperatures on metabolic plasmatic markers in *Sparus aurata*

ABSTRACTS

Plenary Lecture — PL1 (Aula Magna)

Decoding hepatocyte glucose and fatty acid metabolism at the single cell level

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The mammalian liver is composed of parenchymal (hepatocytes) and non- parenchymal (endothelial, Kupffer, cholangiocytes, stellate) cells organized into repetitive hexagonal arrays termed lobules. Within each lobule the hepatocytes are distributed from the portal triad (portal vein, hepatic artery, bile duct) at the six vertices and the central vein located at the centroid of the hexagon. The hepatocytes aligned within a lobule display functionally distinct metabolic properties and are generally divided into three zones: the periportal hepatocytes (zone 1), mid-lobular hepatocytes (zone 2) and the pericentral hepatocytes (zone 3), with emerging single-cell studies indicating that hepatic zonation exists along a more continuous and finely partitioned spectrum. Hepatic gluconeogenesis is active in the fasted state and inactive in the fed state, whereas de novo lipogenesis is active in the fed state and is inactive in the fasted state. However, we previously we reported a remarkable plasticity of hepatocyte gluconeogenic gene expression and gluconeogenic activity across the liver lobule depending upon organismal nutrient state. Using various single cell technologies (scRNA-seq, HCR RNA-FISH, and PrimeFlow), we identified a subset of periportal hepatocytes that simultaneously co-express both gluconeogenic and lipogenic genes in normal physiologic mouse liver. These hepatocytes showed transcriptional metabolic inflexibility, and euglycemic-hyperinsulinemic clamps further demonstrated that this subpopulation is naturally insulin resistant in terms of impaired suppression of gluconeogenic gene Pck1 expression. Spatial metabolomics coupled with stable isotope tracing analyses revealed single hepatocytes that simultaneously undergo both gluconeogenesis and de novo lipogenesis. These hepatocytes were also present in humanized mouse livers. Moreover, hepatocytes that showed transcriptional metabolic inflexibility increased in high-fat diet-fed mice. These results suggest a paradigm shift in our understanding of hepatic glucose and lipid metabolism in that a subset of insulin resistant hepatocytes provides the basis for the development of insulin resistance.

Plenary Lecture — PL2 (Aula Magna)

A vascular–satellite cell axis drives whole-muscle regeneration in adult zebrafish

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Volumetric muscle loss in mammals leads to permanent fibrotic scarring, yet adult zebrafish achieve complete regeneration of 3–4 damaged myomeres within 30 days following caudal peduncle cryoinjury. To dissect the cellular and molecular mechanisms underlying this remarkable capacity, we characterized vascularization dynamics, extracellular matrix remodeling, and myofiber diversity throughout the regenerative process. Expression of the injury-responsive reporter careg, previously established as a marker of tissue plasticity in fin, heart, and retina, within regenerating myomeres suggests that tissue restorative programs are conserved across distinct organ systems in zebrafish. Unexpectedly, careg also marks a specialized sublayer of the slow muscle compartment, revealing previously unrecognized heterogeneity among zebrafish myofiber populations. Vascular network analyses demonstrate that angiogenesis is a critical component of muscle regeneration, and its functional requirement is being tested pharmacologically through inhibition of VEGF receptor tyrosine kinase signaling. Building on the established role of satellite cells in myofiber repair, we propose that endothelial cells constitute an essential component of the satellite cell niche, providing paracrine signals that license regenerative activation. Together, these findings implicate a vascular–satellite cell axis as a central regulator of whole-muscle regeneration and highlight its potential translational relevance to therapeutic strategies for muscle loss in humans.

SOA-1 - Hormonal control of metamorphosis and reproduction in insects: the case of the desert locust

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Most insect species undergo a metamorphic transition from a growing juvenile (larval/nymphal) stage to a reproductive adult stage. The development from a juvenile to a sexually mature individual is tightly controlled and coordinated by functional interactions between the nervous and endocrine systems. Central to this regulation are the *corpora allata* and the prothoracic glands, important endocrine glands which produce the classical lipid-derived hormones juvenile hormone (JH) and ecdysone, respectively. These hormones orchestrate postembryonic development by controlling processes of moulting, metamorphosis, and reproduction. In addition, signals derived from the nervous system regulate the activity of these glands.

In this lecture, I will provide a state-of-the-art overview of the hormonal and molecular mechanisms governing these processes, with a focus on knowledge obtained from studies in a hemimetabolous insect, the desert locust, *Schistocerca gregaria* (Orthoptera). This species remains of major agricultural importance due to its capacity to undergo rapid population expansion and form huge migratory swarms under favourable environmental conditions, causing severe crop losses.

I will highlight recent advances in our understanding of the role of key molecular components, such as Methoprene-tolerant (*Scg*-Met) and Taiman (*Scg*-Tai), which mediate JH signalling, and the Ecdysone receptor (*Scg*-EcR) and Retinoid-X receptor (*Scg*-RXR), which together form the ecdysteroid-responsive nuclear receptor complex. Functional knockdown studies using RNA interference (RNAi) have revealed that disruption of these pathways leads to pronounced defects in development and reproduction, underscoring their essential regulatory roles.

In addition, I will discuss transcriptomic insights into the prothoracic glands, including the identification of genes encoding candidate (neuro)peptide receptors. Potential phenotypic effects of these genes on the timing of the adult moult and on ecdysone biosynthesis in the PG were further investigated for signalling pathways involving the crustacean cardioactive peptide (CCAP), the eclosion hormone (EH), the epidermal growth factor (EGF) and the prothoracicotropic hormone (PTTH).

Finally, I will address the remarkable persistence of the prothoracic glands in adult locusts, in contrast to their degeneration in many other insect species, and present recent findings on the regulation of apoptosis-related processes that may underlie this phenomenon.

Together, these insights provide an updated framework for understanding neuroendocrine mechanisms that control postembryonic development and reproductive maturation, and identify potential molecular targets for innovative strategies in locust management.

This research was supported by the KU Leuven Research Fund (grant number C14/24/076).

O-001 - Kinins, pyrokinins and small molecules in mosquito and tick myoactivity and behavior: towards vector-specific pharmacology

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Through their work and careers, Drs. Angela Lange and Ian Orchard were pioneers in the endocrinology of a model blood feeding arthropod, Rhodnius prolixus, and they inspired a generation of entomologists and biologists to investigate other vectors affecting human and animal health. In my laboratory, a long-term comparative endocrinology approach investigating neuropeptides and cognate receptors in blood feeding vectors offers a unique opportunity to advance knowledge on meal detection, acceptance and ingestion, diuresis, peristalsis, and reproductive output. We selected the female of the mosquito *Aedes aegypti* because is a sporadic feeder that performs in two feeding modalities: ingesting nectar or sugary fluids acquired from plants, and feeding on blood, acquired preferentially from humans, thus their success as vectors of arboviruses. After nectar feeding, primary urine production is regulated mainly by CRF-like secretion (DH_{44}) for cation transport (Na^+ and K^+). However, blood feeding for egg production triggers the release of peptidergic hormonal signals that coordinate digestion, diuresis, and excretion, including calcitonin-like DH_{31} for Na^+ elimination. In both feeding modalities kinins stimulate Cl- transport. Ticks transmit deadly pathogens to humans, wildlife, and cattle. Ticks were selected for being blood feeding parasites, attaching to hosts for several days, and, like *Rhodnius*, acquire a huge blood meal during their fast-feeding phase. We previously cloned, expressed and functionally characterized many of the neuropeptides and cognate GPCRs believed to be involved in post-prandial myotropic function and diuresis such as those for diuretic hormones (DH_{44}, DH_{31}) and kinins in mosquitoes, and those for kinins, periviscerokinins and pyrokinins in ticks. We recently adopted several technologies and tools, adding to the arsenal of insect physiology assays pioneered by Angela and Ian. For example, in ticks, fluorescently-labeled endogenous kinins helped localize the kinin receptor to the midgut muscles. In mosquitoes, kinin peptidomimetics given in sugar meals helped uncover the significant role of the kinin receptor in suppressing mosquito sugar feeding and eliciting an aversive response. However, peptidomimetics are not considered suitable for field applications with toxic sugar baits due to cost and molecule instability, despite modifications using non- natural amino acids. Therefore, towards developing arthropod-specific pharmacology we adapted a high-throughput “dual addition” screening assays for small molecules. With it we identified antagonists of the arthropod kinin receptor that paralyzed the mosquito ex vivo hindgut and tick midgut. We also adapted an electronic flyPAD device to investigate the individual female mosquito feeding behavior in high-throughput mode. Using this, we found that a synthetic small molecule antagonist of the kinin receptor increased sugar feeding. Further, when added with the insecticide malathion increased not only the volume ingested by 27% but also female mortality of this sugar bait. A potent kinin peptidomimetic that prevented sugar feeding also decreased blood feeding when added to a blood meal, overriding the phagostimulant effect of 1mM ATP added to the meal. Finally, through high-throughput screening, we found an antagonist “hit” on the tick kinin receptor. This molecule and a structural analog were acaricidal to susceptible and pyrethroid-resistant cattle tick larvae. While later it was proven not to be an antagonist of the kinin receptor, and likely acting through another GPCR, the approach is promising to identify new molecules and targets for tick control.

O-002 - Neuropeptides controlling diuresis also regulate reproductive processes in female, *Rhodnius prolixus*.

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Rhodnius prolixus is a blood-feeding hemipteran widely used as a model for studying physiological changes following a blood meal. In adult females, blood feeding triggers both rapid post-prandial diuresis and egg production through the actions of multiple neuroendocrine signaling pathways. The corticotropin-releasing factor-like diuretic hormone, Rhopr-CRF/DH, is released from lateral neurosecretory cells (NSCs) of the mesothoracic ganglionic mass (MTGM) during blood gorging and acts synergistically with serotonin to promote diuresis, whereas Rhopr-CAPA-2, one of three CAPA peptides encoded by the capability gene and released from midline NSCs of the MTGM, functions as an anti-diuretic hormone. These NSCs also express the glycoprotein hormone GPA2/GPB5, a thyrostimulin-like heterodimeric hormone that signals through the receptor LGR1. Recently, in R. prolixus, GPA2/GPB5 signaling has been shown to regulate reproduction by delaying oogenesis until sufficient nutrients are available. Thus, knockdown of LGR1 accelerates oogenesis and increases both egg number and egg size, suggesting that GPA2/GPB5 acts as a gonad-inhibiting hormone. The transcripts of the receptors for Rhopr-CRF/DH (Rhopr-CRF/DH-R2) and for Rhopr-CAPA-2 (Rhopr-CAPA-2-R) are also expressed in female reproductive tissues and fat body (FB), with transcript abundance increasing after a blood meal. We therefore investigated whether Rhopr-CRF/DH and Rhopr- CAPA-2 also participate in the control of egg production. RNA interference experiments revealed opposing reproductive functions for these two pathways. Downregulating Rhopr-CRF/DH-R2 increased transcript levels of the major yolk protein RhoprVg1 in both FB and ovaries, elevated transcript expression of the vitellogenin receptor RhoprVgR, accelerated oogenesis, and increased egg production. ELISA also showed elevated Vg levels in both the FB and hemolymph. In contrast, knockdown of Rhopr-CAPA-2-R reduced RhoprVg1 and RhoprVgR transcript expression, decreased Vg content in the FB and hemolymph, and reduced the number of eggs produced. These findings demonstrate that Rhopr-CRF/DH acts as a gonad- inhibiting hormone, whereas Rhopr-CAPA-2 functions as a gonadotropin, coordinating reproductive timing with the physiological demands of blood feeding. Together with the gonad-inhibiting effects of GPA2/GPB5, these neuroendocrine pathways form a complex regulatory network that integrates nutrient availability, osmoregulation, and reproductive output in adult female *R. prolixus.*

Funding: This work was supported by Discovery Grants from the Natural Science and Engineering Research Council of Canada (NSERC) to ABL and IO, and NSERC PGS Doctoral Scholarship to ANA.

O-003 - The integration of multiple modulatory pathways controls juvenile hormone synthesis in insects

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Juvenile hormones (JH) are sesquiterpenoids synthetized in the *corpora allata* (CA). The CA biosynthetic activity is regulated by developmental transitions, mating, nutrition, circadian rhytms and other environmental and physiological factors. The regulation of CA activity is very complex, as the CA integrates multiple stimulatory and inhibitory signals to determine the final synthesis rate. The allatoregulatory molecules that control JH synthesis include ecdysteroids (20E), ecdysis triggering hormone (ETH), insulin, allatostatin (AST-C), neuropeptide F (NPF) and diurretic hormone 31 (DH31). In this presentation, we will discuss the recent advances in our understanding of the allatoregulatory molecules that control CA activity in insects.

O-004 - Reproductive functions of Diuretic hormone 44 in Drosophila

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Diuretic hormone 44 (Dh44) is the Drosophila ortholog of vertebrate corticotropin-releasing hormone (CRH), sharing sequence homology and Gαs–cAMP–PKA–CREB signaling through its two GPCRs, Dh44R1 and Dh44R2. While mammalian CRH is best known as the apical coordinator of the stress axis, our work reveals that Dh44 has been repeatedly co-opted to orchestrate female reproduction in Drosophila melanogaster. I will integrate findings from three studies that define Dh44 as a central node linking internal state to reproductive output.

First, six Dh44 neurons in the pars intercerebralis (Dh44-PI) control sperm ejection timing or the ejaculate-holding period and sperm storage. Suppressing Dh44 or Dh44R1 in doublesex^{+} neurons causes precocious ejaculate expulsion, depletes stored sperm, and impairs post-mating responses (Lee et al., Curr. Biol. 2015).

Second, males transfer a phospho-galactoside, "venerose," in their seminal fluid. Venerose enters the hemolymph and activates Dh44-PI neurons via the sugar transporter Tret1-1. Released Dh44 stimulates germline stem cell proliferation through Dh44R2 in niche cells, and prolongs the ejaculate-holding period to enhance venerose absorption. Energy-deprived females retain a larger releasable Dh44 pool and therefore respond more strongly to venerose, enabling them to bias sperm storage according to the amount of venerose transferred by males (Kim et al., Nat. Commun. 2024).

Third, Dh44 acts as an autocrine signal within doublesex^{+} pC1 neurons gating sexual drive. A Dh44–Dh44R–CREB–Pyrexia feedback loop progressively elevates pC1 excitability during sexual maturation; conversely, sexual drive is suppressed in mated females by Sex peptide suppressing Dh44 secretion (Kim et al., PNAS 2024).

Together, anatomically distinct Dh44–Dh44R modules orchestrate sperm handling, germline stem cell dynamics, and sexual motivation, suggesting under-appreciated reproductive roles for vertebrate CRH peptides.

O-005 - Blood meal–induced juvenile hormone signaling drives male reproductive maturation in the classical insect model *Rhodnius prolixus*

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Juvenile hormone (JH) is a key endocrine regulator coordinating development, physiology, and reproduction in insects. Neuroendocrine signaling plays a central role in integrating feeding status with reproductive physiology and behaviour, a coordination particularly well described in hematophagous insects. In adult males, however, the role of JH signaling during reproductive maturation and reproductive behaviours remains poorly understood. Using the Chagas disease vector Rhodnius prolixus as a model, we investigate how JH signaling regulates maturation of the male reproductive system following a blood meal. In R. prolixus males, the transparent accessory glands (TAG) produce seminal fluid proteins that are transferred during copulation and influence female post-mating physiology and reproductive behaviour. Previous studies suggested that the accumulation of proteins in TAG following feeding is regulated by JH. Here, we examined the dynamics of JH signaling and TAG maturation after feeding. We observed an increase in JH titers in male hemolymph after a blood meal, accompanied by a significant rise in protein accumulation in TAG. Temporal transcript expression analyses revealed that key components of the JH signaling pathway, the nuclear JH receptor Methoprene-tolerant (Met) and its cofactor Taiman (Tai), are present in TAG, while the downstream target gene Krüppel homolog 1 (Kr-h1) is significantly upregulated after feeding. Functional knockdown of Met using RNA interference resulted in a significant reduction in TAG size and protein content at 2 and 4 days post-feeding, supporting a role for JH signaling in regulating accessory gland growth. These findings suggest that JH signaling plays a central role in coordinating feeding-dependent endocrine signals with maturation of the male reproductive system and the production of seminal factors that influence reproductive interactions.

O-006 - Analysis of neuropeptidergic coupling factors controlling multiscale behavioral rhythms in *Drosophila melanogaster*

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The diurnal fruit fly, *Drosophila melanogaster*, exhibits circadian and ultradian rhythms in rest and activity. Its activity peaks at dawn and dusk. As well as having circadian feeding patterns, fruit flies also display ultradian feeding bouts that vary according to food availability and quality. The interaction between these circadian and ultradian rhythms remains to be elucidated. It has been demonstrated that starvation and nutrient restriction alter sleep and activity patterns, a phenomenon likely mediated by neuropeptides that coordinate multiscale rhythms. We hypothesize that neuropeptides released by clock neurons oscillate on multiple timescales, thereby integrating behavioral rhythms into a unified framework. In this study, we examine the role of the ring gland in synchronizing neuropeptide release from the pars intercerebralis and SEG neurons with circadian timing using a combination of mass spectrometry, behavioral assay and computational neurochemistry. Experiments were conducted on individual larvae under various nutritional conditions (fed, starved for 24 or 48 hours) and zeitgeber times (ZT0–ZT23). The larvae exhibited daily oscillations, peaking at dawn, dusk, midday, and midnight. Foraging behaviors showed significant modulation in response to starvation, with alterations occurring at specific times. Semiquantitative MS analysis revealed significant variations in neuropeptide abundance, including e.g. increases in AKH and decreases in CAPA-PK at different ZTs, with alterations in phase and amplitude. Profiling the spatial distribution of neuropeptides within the brain by imaging MS in relation to distinct physiological states, such as time-of-day-dependent changes, will provide a more detailed insight into the mechanisms that govern the formation and maintenance of circadian and ultradian rhythms.

O-007 - RYamide signaling controls selective ion reabsorption by the hindgut in the yellow fever mosquito, *Aedes aegypti*

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Neuropeptide Y-related signaling, involving diverse neuropeptides including mammalian neuropeptide Y (NPY), pancreatic polypeptide (PP), and peptide YY (PYY) families, along with neuropeptide Y-like peptides in insects, are evolutionarily ancient and occur across vertebrates and invertebrate organisms. A more recently discovered NPY-related neuropeptide family in arthropods are the RYamides. To date, studies on insect RYamides have primarily focused on their roles in reducing feeding motivation, including the suppression of host-seeking behaviour in female mosquitoes. Here, we highlight recent developments in profiling RYamide expression and localization in the nervous system and peripheral organs, deorphanizing two RYamide receptors and analyzing their evolutionary origins across bilaterians, and elucidating roles of RYamide signaling in ionoregulatory activity by the hindgut of this important human disease vector, Aedes aegypti. Phylogenetic analysis revealed that, despite most arthropods retaining only a single RYamide receptor, a subset of mosquito species have two RYamide receptors, suggesting potential subtype-specific functional specialization. This is also true for our study organism, Ae. aegypti, in which we identified two orphan G protein-coupled receptors through heterologous expression as bona fide RYamide receptors that exhibit highly selective ligand activation in the pico- to nanomolar range. Abundant RYamide expression and strong immunoreactive staining was observed in the nervous system, including in the terminal abdominal ganglion of the ventral nerve cord. In parallel to our work, the RYamide receptor 2 (RYaR2) was recently localized to the rectal pads within the posterior hindgut (rectum) of the mosquito, supporting a close association necessary for RYamide- RYaR2 signaling regulating hindgut-related functions. Indeed, our latest results demonstrate that RYamides function as ionoregulatory factors, as evidenced by increased K^+ reabsorption across the hindgut of wild-type mosquitoes treated with RYamides, whereas RYaR2-knockout mosquitoes showed no response to RYamide treatment. Comparatively, unlike the observed effects on K+ flux, our data revealed that Na+ transport across the rectal pads is not influenced by RYamides. Interestingly, while RYamide-RYaR2 signaling in female mosquitoes has been suggested to facilitate nutritional sensing and communication of this sensory information to the nervous system to regulate reproductive physiology, our data indicate that K+ reabsorption by the rectal pads is increased following RYamide treatment in both male and female mosquitoes. Collectively, these novel findings reveal RYamides and RYaR2 signaling participates in hindgut-related physiology necessary for ionic homeostasis in adult mosquitoes. In ongoing work, we are examining how disruption of RYamide-RYaR2 signaling might influence longevity and sensitivity to desiccation stress and measuring both water content and ion levels in the haemolymph as a proxy for hydromineral homeostasis in mosquitoes. Maintenance of hydromineral balance is critical for all organisms, but for mosquitoes like Ae. aegypti, interfering with neuropeptide signaling that controls water and salt homeostasis could impact the survival of these disease vectors under adverse environmental conditions helping to reduce their worldwide disease risk given they transmit several viral diseases.

SOA2 - Beyond calcium regulation: New roles for Stanniocalcins in growth, hypoxic stress, and disease

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This presentation will discuss our evolving understanding of the stanniocalcin/STC glycoprotein family, with a focus on the distinct functions and regulation of STC1 and STC2. STC1 was first discovered in the 1970s as a calciotropic hormone in fish. Once thought to be fish-specific, STC1 is now recognized as a multifaceted glycoprotein with deep roots in metazoan evolution, important functions in mammals, and significant implications for human health. In humans and other mammals, STC1 and STC2 are expressed locally in many tissues and act through complex mechanisms, including interactions with the insulin-like growth factor (IGF)-IGF binding protein signaling pathway. Dysregulation of STC1 and STC2 expression has been associated with multiple types of cancer and other human diseases. Recent genetic and mechanistic studies in zebrafish have provided new insight into the diversification of STC function. Zebrafish possess multiple stc genes, each with distinct spatiotemporal expression patterns and regulatory mechanisms. Among the four zebrafish stc genes, stc1a is expressed in the corpuscles of Stannius and is regulated in a Ca^{2+} concentration-dependent manner. Genetic and mechanistic studies show that Stc1a is essential not only for regulating calcium uptake and bone mineralization, but also for controlling the fate of calcium-transporting cells, as well as brain and sensory neuron function. In contrast, stc2a is expressed primarily in the adult brain and is upregulated in response to hypoxia. Loss of stc2a accelerates development and growth, resulting in increased adult organ and body size. Under hypoxic conditions, stc2a mutant fish grow faster than wild-type fish but exhibit reduced survival. These phenotypes are reversed by inhibition of IGF signaling, suggesting that Stc2a limits IGF-mediated somatic growth to promote survival under stress. Together, these findings reshape the view of the STC family and demonstrate that Stc1a and Stc2a have distinct physiological functions. By synthesizing these recent discoveries, this talk will offer a new framework for understanding STC function and regulation across evolution, physiology, and disease.

O-008 - Somatostatin regulation of the pancreatic β-cell proliferation

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Understanding the regulation of pancreatic β-cell proliferation is crucial for comprehending the pathogenesis and treatment of diabetes. In a previous study, we found that somatostatin 1.2 (*sst1.2*)-deficient mutants displayed both a germ cell and pancreatic β-cell overproliferation phenotype, suggesting a possible role in regulating energy allocation during reproduction. Analysis of the sst1.2 promoter revealed enrichment with binding sites for Hoxb8a, with expression of *hoxb8a* transcripts located in β and δ cells in 5-dpf zebrafish. Both knockdown and knockout of *hoxb8a* showed a β-cell over-proliferation phenotype analogous to that of the sst1.2 mutant. A thymidine incorporation assay confirmed that both sst1.2 and hoxb8a regulate β-cell mass via cell proliferation. The *sst1.2*/*hoxb8a* double mutant also had an increase in β-cell mass. In the *hoxb8a* knockout, *sst1.2* was downregulated compared to the wild-type, and Hoxb8a promoted the transcription of *sst1.2* in a luciferase assay.

To further elucidate the downstream gene regulation mediated by sst1.2 and hoxb8a, we analysed Nrf2, which has been found to regulate β-cell mass in mice. We localised *nrf2a* mRNA in β cells in 5-dpf zebrafish and found upregulation of *nrf2a* mRNA and nrf2/pnrf2 protein in both *sst1.2* and *hoxb8a* mutants. The zebrafish *nrf2a* gene knockout showed reduced β-cell mass.

To investigate the crosstalk between β and δ cells, we analysed the protein kinases Akt and AMPK, which are important, respectively, for cell growth and survival, and as energy sensors. We found decreased protein levels of Akt and pAkt in both *sst1.2* and *hoxb8a* mutants, including in β-cells. In parallel, AMPK and pAMPK protein levels were upregulated in both *sst1.2* and *hoxb8* mutants, including the pancreatic β cells. In summary, hoxb8a regulates sst1.2, which targets nrf2a to limit β-cell proliferation via protein kinases pAKt and pAMPK.

In conclusion, our study offers novel insights into the regulation of somatostatin-mediated β-cell proliferation.

Acknowledgement: This work was funded by the Shanghai Municipal Government to Shanghai Ocean University and by the FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

O-009 - Novel regulators of HIF signaling in zebrafish hypoxia stress model

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The hypoxia-inducible factor (HIF) signaling pathway is central to cellular and organismal hypoxic adaptation, critically regulating hematopoiesis, metabolic reprogramming, and the progression of multiple hypoxia-associated disorders. The canonical PHD-pVHL pathway responsible for HIF-α degradation under normoxia is well-established, whereas non-canonical mechanisms fine-tuning HIF activity remain largely unelucidated. Employing zebrafish as a robust *in vivo* hypoxia model, our group has focused on exploring unreported HIF regulatory factors and previously identified three distinct regulators (VBP1, MED15, and AKAP17A) that modulate HIF signaling via unique mechanisms. Our recent work further characterizes Microrchidia family CW-type zinc-finger 2 (MORC2) as a novel negative regulator of HIF-1α. *In vivo*, zebrafish morc2 knockout leads to systemic hypoxia, polycythemia, and constitutive HIF pathway hyperactivation. Mechanistically, MORC2 competitively interacts with HIF-1α to hinder HDAC4 recruitment. MORC2 deficiency facilitates HDAC4-mediated deacetylation of HIF-1α at the lysine 629 residue, which prevents HIF-1α proteasomal degradation and sustains persistent HIF signaling activation. This newly identified MORC2-HDAC4 deacetylation axis reveals an unrecognized epigenetic mechanism controlling HIF-1α stability, effectively complementing and enriching the non-canonical HIF regulatory network. These *in vivo* findings advance the mechanistic understanding of organismal hypoxic adaptation and offer valuable molecular targets for the treatment of HIF-related hematological and hypoxic disorders.

O-010 - Gcn2-mediated nutrient sensing orchestrates metabolic homeostasis and hypoxic tolerance in zebrafish

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The Integrated Stress Response (ISR) is a highly conserved cellular signaling mechanism essential for maintaining metabolic homeostasis and adapting to physiological stressors during development. In teleosts, the precise metabolic networks linking nutrient sensing to developmental growth and stress adaptation remain to be fully elucidated. General Control Nonderepressible 2 (GCN2), an evolutionarily conserved eIF2α kinase, functions as a primary sensor for amino acid availability. Using a *gcn2* knockout zebrafish model, we investigated the *in vivo* role of GCN2 in regulating stress adaptation and systemic homeostasis. We found that Gcn2 deficiency profoundly impairs hypoxia tolerance and triggers metabolic reprogramming, which is characterized by altered tricarboxylic acid cycle intermediates and elevated lactate levels. Strikingly, *gcn2* mutants exhibit severe embryonic anemia with significantly reduced erythrocyte numbers and hemoglobin expression. This anemic phenotype is accompanied by the upregulation of erythropoietin (*epoa*) and *igfbp1b* expression, indicating a compensatory endocrine and growth factor response to developmental and hypoxic stress. Mechanistically, the loss of Gcn2 disrupts intracellular iron and redox homeostasis, leading to elevated free ionic iron, reactive oxygen species accumulation, and excessive heme degradation via Hmox1 upregulation. We further demonstrate that Gcn2 tightly regulates cysteine transport and glutathione biosynthesis through the eIF2α-Atf4-Slc3a2b signaling axis. The disruption of this specific amino acid transport system in *gcn2* mutants induces lipid peroxidation and ferroptosis, which directly contribute to the observed erythrolysis and hypoxic intolerance. The ferroptotic and anemic phenotypes, as well as the hypoxia-induced mortality, could be effectively mitigated by pharmacological supplementation with iron chelators, antioxidants, and ferroptosis inhibitors. Collectively, our findings identify the Gcn2-mediated nutrient-sensing pathway as a critical gatekeeper of redox homeostasis and metabolic balance in zebrafish. By integrating amino acid availability with systemic oxygen transport and growth factor signaling, GCN2 emerges as a pivotal regulator of developmental growth and physiological adaptation in aquatic vertebrates.

O-011 - The endocrine role of bone in gilthead sea bream (*Sparus aurata*): systemic effects of BMP7 and SPARC

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Bone is recognized as an endocrine organ in mammals involved in inter-tissue communication through the secretion of bone- derived proteins (a.k.a. osteokines). In fish, the autocrine and endocrine effects of osteoblast-conditioned medium (OCM) have been demonstrated in different *in vitro* models derived from the same species, including primary osteoblasts, adipocytes, myocytes, as well as the SAEC-H7 embryonic stem cell line. In addition, proteomic characterization of the OCM identified hundreds of secreted bone-derived factors; however, the physiological effects of these proteins in fish remain largely unexplored.

To elucidate the potential functions of some of these factors as osteokines, eight proteins were selected from the ones identified in the OCM secretome and evaluated in the SAEC-H7 cell line at different concentrations. Cell viability, proliferation and several gene transcripts levels were analyzed. Based on the results obtained two proteins were used for a subsequent study *in vivo*. Commercially available human recombinant Bone morphogenic protein 7 (BMP7) and Secreted protein acidic and cysteine rich (SPARC) were diluted in phosphate buffered saline (PBS) and intraperitoneally injected into gilthead sea bream juveniles (~10 g) at a dose of 100 ng/g body weight. Control fish were injected with the same volume of PBS. Fish from the three experimental groups were maintained in separate cages within the same tank and then sampled at 6 h and 24 h post-injection. Liver, muscle, bone, and whole brain were collected from fourteen fish per group, and the mRNA levels of genes related to growth, differentiation and metabolism were determined.

BMP7 and SPARC did not significantly affect the viability and proliferation of SAEC-H7 cells, although SPARC tended to increased viability, and was the only protein among those evaluated showing a trend toward increased cell proliferation (determined as BrdU positive cells). The number of nuclei was also analyzed as an additional cellular parameter, and BMP7 treatment at 100 ng/mL significantly increased this parameter. Following intraperitoneal injection, neither BMP7 nor SPARC affected fish survival. Furthermore, although gene expression analyses are still ongoing; preliminary results from skeletal muscle show that BMP7 reduced the expression of the proliferation marker *pcna* and the myogenic progenitor marker *pax7*, as well as increased the expression of the myogenic regulatory factor *myod1* at 6 h post-injection, thus indicating a potential pro-differentiation effect *in vivo*. On the other hand, SPARC administration increased the expression of the inflammatory marker *tnfα*, the pro-apoptotic marker *bax*, and the cell cycle regulator *hes1-b*, while *myod2* mRNA levels were downregulated, suggesting a role in maintaining the cells in an undifferentiated state. However, at 24 h post-injection, BMP7 did not affect the expression of any of the genes analyzed, whereas fish injected with SPARC showed increased expression of *bax*, *pcna* and the growth factor *igf1*. This suggests that this osteokine plays a stronger role in proliferation or tissue remodeling than in differentiation, which is consistent with previous *in vitro* results. Overall, these data provide the first evidence that osteoblast-derived osteokines may exert systemic effects in fish, potentially influencing muscle growth, and highlighting the importance of bone in regulating growth, development, and tissue regeneration.

Funded by projects PID2020-116172RB-I00 and PID2024-161415OB-I00 (MICIU/AEI/10.13039/501100011033 and ERDF/EU. IR is supported by fellowship (PRE2021-100391) from MICIU/AEI/10.13039/501100011033 and ESF+. DGS is a Serra Húnter Associate Professor.

O-012 - Role of the venus kinase receptor in the nymphal growth of the desert locust, *Schistocerca gregaria*

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Venus kinase receptors (VKRs) are a subfamily of invertebrate receptor tyrosine kinases (RTK), which consist of an intracellular tyrosine kinase (TK) domain similar to that of the insulin/insulin-like peptide receptor (IR/InR) and an extracellular Venus FlyTrap (VFT) domain. VKRs were originally discovered in the platyhelminth parasite *Schistosoma mansoni* in 2003 [1] and further functionally characterized in the mosquito *Aedes aegypti* [2], the desert locust *Schistocerca gregaria* [3], and the mosquito *Anopheles coluzzii* [4], in which VKRs play a crucial role in female reproduction or larval growth and immunity. Here we characterize the role of a VKR in nymphal growth in *S. gregaria*. *SgVKR* transcript levels throughout the fifth nymphal instar in different tissues were profiled, and its possible role in the growth process was investigated using RNA interference. *SgVKR* knockdown significantly slowed down the body weight gain and the onset of nymphal to adult ecdysis. Hemolymph ecdysteroid levels and the expression of other related genes were affected as well. Our work firstly reports the role of VKR in growth of nymphal locusts, which provides a valuable insight in the deeper understanding of the function of VKRs.

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Acknowledgements:

The authors acknowledge Evelien Herinckx, Arnold Van Den Eynde, Lore Smekens, and Marijke Christiaens for their technical assistance in the locust breeding and lab maintenance. The authors are also very grateful to the master student Aaron Claes for his practical assistance in conducting experiments. The authors gratefully acknowledge China Scholarship Council (CSC) and KU Leuven Research Foundation (C14/24/076) for funding.

O-013 - Cellular and molecular dynamics underlying the transition from hyperplastic to hypertrophic muscle growth in rainbow trout

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Postnatal skeletal muscle growth differs markedly among vertebrates. While mammals predominantly rely on fiber hypertrophy after birth, large teleost fish such as rainbow trout (*Oncorhynchus mykiss*) maintain an extensive capacity for muscle fiber hyperplasia during juvenile growth. However, the mechanisms underlying the progressive decline of hyperplasia and the transition toward hypertrophic growth remain poorly understood. Here, we investigated the respective contributions of muscle stem cells and their niche to this transition using histological, functional, and single-cell transcriptomic approaches. Histological analyses performed in trout ranging from 10 g to 500 g revealed a progressive reduction in hyperplastic activity, estimated by the proportion of fibers smaller than 25 µm, together with a decline in Pax7$^+$ satellite cell abundance. To assess whether this reduction reflected intrinsic alterations of muscle progenitors and/or changes in their microenvironment, we performed transplantation experiments using muscle-derived cells from *mlc2*-GFP transgenic trout transplanted into recipients of different growth stages. These experiments demonstrated a marked decline in both the intrinsic myogenic capacity of progenitors and the functionality of the muscle niche as fish growth progressed from 10 g to 500 g. Detailed analyses of donor-derived GFP$^+$ fibers showed a strong enrichment of small newly formed fibers in 10 g recipients but not in 100 g recipients, indicating an early impairment of the tissue environment supporting hyperplastic growth. Furthermore, transplantation of cells from trout of different ages but identical body weights demonstrated that increased body weight, rather than chronological aging, is primarily associated with the decline in progenitor function and niche functionality. To further characterize the cellular and molecular mechanisms associated with the transition from hyperplastic to hypertrophic growth, we generated a single-cell transcriptomic atlas of muscle-derived cells across five juvenile growth stages. Fifteen tissue-resident cell populations were identified, including eight myogenic subpopulations spanning from quiescent satellite cells to terminally differentiating myocytes. RNA velocity analyses uncovered two distinct transcriptional trajectories: one specifically associated with hyperplastic growth and another maintained throughout growth, suggesting the existence of specialized muscle stem cell fates linked to hyperplasia or hypertrophy. Comparative analyses with human single-cell atlases revealed similarities between trout myogenic subpopulations and stage-specific fetal or adult human myogenic states. Notably, we identified a pax7$^+$/pdgfrα$^+$ cell population displaying features of fibroblastic lineage plasticity and associated with hypertrophic growth, highlighting a potential role for mesenchymal interactions in muscle remodeling. In parallel, extracellular matrix remodeling accompanied growth progression, supporting the existence of a dynamic crosstalk between myogenic and mesenchymal compartments. Together, these findings demonstrate that the decline of muscle hyperplasia in trout results from coordinated intrinsic and extrinsic alterations affecting both muscle stem cells and their niche. Our work identifies distinct myogenic programs associated with hyperplastic and hypertrophic growth and provides new insights into muscle stem cell plasticity and niche remodeling during postnatal skeletal muscle growth.

O-014 - Identification of ohnologues transcriptome networks involved in myogenic differentiation of the Atlantic salmon

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Salmonids are among the highly valued species in global aquaculture and a paradigm to study genome evolution. Like other teleosts, muscle is the most abundant tissue in salmonids, constituting ~60% of body mass. Myogenesis, the process by which progenitor muscle cells (MPCs) differentiate into large, multinucleated myofibres, is a highly conserved and well- orchestrated developmental process in vertebrates. Unlike mammals, salmonids demonstrate indeterminate growth through continuous production of muscle fibre (hyperplasia) into adulthood. Throughout vertebrate evolution, whole genome duplication (WGD) events have restructured gene networks and cell developmental programs. After the teleost-specific WGD (Ts3R) event, salmonids underwent another round of WGD (Ss4R) about 100 million years ago, which established a repertoire of ohnologs with subsequent capacity to evolve and acquire new functions. Here, using single-nucleus RNA-seq study of epaxial fast muscle in Atlantic salmon, we have determined eight distinct differentiation states from quiescent MPCs through to terminal myofiber cells. We present evidence for expression divergence among ohnologs of master myogenic regulatory factors, capturing evolutionary changes in the transcriptional program of myogenesis following the Ss4R. Additionally, we provide evidence for distinct gene co-expression networks functioning in hyperplasia and hypertrophy stages of myogenesis. Our study establishes an initial foundation of cellular heterogeneity during myogenesis in salmonid fishes at the single-cell level, and especially in the context of evolutionary alterations following the Ss4R WGD.

O-015 - Physiological, transcriptional and epigenetic response in rainbow trout (*Oncorhynchus mykiss*) skeletal muscle mediated by chronic stress and infectious pancreatic necrosis virus (IPNV)

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INTRODUCTION: Stress is a highly conserved physiological response that allows vertebrates to adapt to environmental and biological challenges. In teleost fish, chronic activation of the stress axis promotes sustained cortisol release, affecting metabolism, immune function, and growth. Skeletal muscle, the largest tissue in fish and a key regulator of locomotion and energy balance, is particularly sensitive to chronic stress and pathogen-induced alterations. Infectious pancreatic necrosis virus (IPNV), an important salmonid pathogen, has been mainly studied in classical immune tissues, while its long-term effects on skeletal muscle remain poorly understood. Moreover, the influence of chronic stress on the transcriptional and epigenetic responses induced by viral infection has not been characterized. Since epigenetic mechanisms such as DNA methylation may mediate long-term cellular adaptations, this study evaluated the effects of chronic stress induced by cortisol implants or crowding on the physiological, transcriptomic, and epigenetic responses triggered by IPNV infection in rainbow trout skeletal muscle. METHODS: Juvenile rainbow trout were exposed for 14 days to chronic stress through two independent protocols: intraperitoneal cortisol implants (10 mg/kg) or high stocking density (crowding). After the stress period, fish were experimentally infected with IPNV and maintained for 30 days post-infection. Control groups included non-stressed and non-infected fish. Plasma and skeletal muscle were obtained, frozen in liquid nitrogen and then stored at -80°C for subsequent analysis. Plasma levels of cortisol, glucose, and lactate were quantified using commercial kits. RNA was extracted from the skeletal muscle using Qiagen RNeasy Mini Kit of control and treated groups and analyzed by RNA-sequencing (RNA-seq). Similarly, Genomic DNA was extracted using Qiagen DNeasy Blood & Tissue kit and analyzed by Whole Genome Bisulfite Sequencing (WGBS). RESULTS & DISCUSSION: Chronic stress exposure significantly altered the physiological condition of rainbow trout, showing reduced growth performance and altered metabolic indicators. Transcriptomic analyses revealed that IPNV infection induced important changes in genes associated with inflammatory responses, cytoskeleton organization, proteolysis, and transcriptional regulation in skeletal muscle. Interestingly, fish previously subjected to cortisol implants or crowding displayed an exacerbated transcriptional response to IPNV infection, including the differential regulation of genes involved in immune signaling, oxidative stress, extracellular matrix remodeling, and muscle atrophy pathways. Epigenomic analyses identified extensive DNA methylation changes associated with chronic stress and viral infection, particularly in genes related to transcriptional regulation, chromatin organization, and muscle development. Integrative analyses suggested that chronic stress modulates the skeletal muscle response to IPNV through epigenetic mechanisms capable of altering transcriptional plasticity during infection. Moreover, several genes involved in growth regulation and immune function exhibited coordinated differential expression and methylation patterns, supporting the existence of stress-mediated epigenetic programming in fish skeletal muscle. Overall, these findings provide new insights into the molecular mechanisms through which chronic stress influences host-pathogen interactions in salmonids and contribute to the understanding of how intensive aquaculture conditions may affect fish health and productive performance.

This work was supported by Agencia Nacional de Investigación y Desarrollo, ANID INCAR2 CIA250009 and Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) grant 1230794.

SOA3 - Prebilaterian origin of monoaminergic signalling

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Monoamines regulate diverse physiological and behavioural processes in bilaterian animals, but their evolutionary origin remains unclear. Placozoans are neuronless, non-bilaterian animals that exhibit coordinated behaviours, making them a key lineage for testing whether monoaminergic signalling predates bilaterian nervous systems. Here, we combined receptor deorphanisation, phylogenetics, metabolomics, heterologous complementation, expression analysis, and behavioural assays to investigate monoaminergic signalling in Trichoplax adhaerens. We identified five placozoan GPCRs activated by tyramine, tryptamine, or phenethylamine. Four of these receptors are homologous to bilaterian melatonin receptors, revealing an evolutionary link between placozoan monoamine receptors and a major bilaterian monoaminergic receptor family. Targeted LC–ESI–MS/MS detected tyramine, tryptamine, and phenethylamine endogenously in placozoans, while heterologous complementation in Caenorhabditis elegans showed that the T. adhaerens aromatic amino acid decarboxylase can restore tyramine-dependent behaviour. Hybridisation chain reaction revealed spatial expression of AADC and monoamine receptors in distinct epithelial and fibre-cell-associated domains. Finally, exogenous monoamines produced reversible changes in locomotion and body shape. Together, these findings demonstrate that placozoans possess monoamine synthesis, receptor-mediated detection, and monoamine-responsive behaviours, supporting a prebilaterian origin of core monoaminergic signalling components.

O-016 - Neuropeptide signalling in placozoa

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Placozoans are flat, ciliated non-bilaterian animals that lack synaptically connected neurons. However, these animals possess diverse neurosecretory cells and a large number of neuropeptides and other transmitter molecules. Based on large- scale deorphanisation and single-cell sequencing data we mapped the multilayer peptidergic connectome in placozoa. Behavioural experiments revealed that some neuropeptides can elicit long-term oscillations characterised by cycles of flattening and shrinking. By pharmacological treatments we dissect the signalling pathways involved to understand the dynamics of these peptide-induced behaviours. Placozoa are an excellent model to study purely chemical signalling in animal behaviour, without modulation by synaptic signalling.

O-017 - Regulation of simple metamorphosis in insects following the discovery of the Chinmo factor

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Postembryonic development in insects can be classified into three major developmental modes: ametaboly (absence of metamorphosis), hemimetaboly (simple metamorphosis), and holometaboly (complete metamorphosis). In hemimetabolan insects, nymphs resemble miniature adults and gradually acquire adult features through successive molts. In contrast, holometabolan insects undergo a larval stage that is morphologically distinct from the adult and complete their development through a pupal stage before the imaginal transformation. In both developmental modes, metamorphosis is primarily regulated by two hormones, 20-hydroxyecdysone (20E) and juvenile hormone (JH), which control the expression of key genes governing morphogenetic transitions. In hemimetabolan insects, 20E promotes molting, whereas JH prevents metamorphosis through a relatively simple molecular mechanism. During juvenile development, JH induces the expression of the gene *Krüppel homolog 1* (*Kr-h1*) through its receptor Methoprene-tolerant (Met). Kr-h1, in turn, represses the expression of the *E93* gene, whose product triggers adult morphogenesis. *E93* expression depends on 20E signaling through the ecdysone receptor (EcR). Consequently, the presence of JH and the resulting expression of *Kr-h1* maintain *E93* repression during juvenile development, thereby preventing premature metamorphosis. This regulatory arrangement allows the insect to reach the critical size required for successful adult development. During the final nymphal instar, JH levels decline to nearly undetectable levels, leading to the cessation of Kr-h1 expression, activation of E93, and the onset of adult morphogenesis. This regulatory cascade is known as the MEKRE93 pathway.

Recently, a new factor involved in the regulation of metamorphosis has been identified: Chinmo (Chronologically Inappropriate Morphogenesis). Described by Truman and Riddiford in 2022 in relation to the metamorphosis of the fruit fly *Drosophila melanogaster*, Chinmo was shown to repress both *E93*, which specifies adult development, and *Broad-Complex* (*BR-C*), which specifies pupal development. In hemimetabolan insects, Chinmo also represses *E93*, whereas no effect on *BR- C* has been detected, consistent with the absence of a pupal stage in these insects. The repressor role of Chinmo on *E93* has been demonstrated in the German cockroach *Blattella germanica* (results from our laboratory) and in the hemipteran *Oncopeltus fasciatus* (results from the laboratory of Yui Suzuki). Interestingly, depletion of either Chinmo or Kr-h1 independently induces precocious metamorphosis in both hemimetabolan models. These observations indicate that both Chinmo and Kr-h1 are required simultaneously to repress *E93* and, consequently, to prevent metamorphosis. The incorporation of Chinmo into the MEKRE93 pathway adds another layer of control within this regulatory axis, which governs insect metamorphosis, in general. By requiring the simultaneous presence of both Kr-h1 and Chinmo for *E93* repression, the MEKRE93 pathway gains greater specificity, robustness, and context dependence, reducing the likelihood of inappropriate activation of the metamorphic program.

O-018 - A mating responsive hormonal program supports female reproductive success during periods of dietary restriction

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Reproduction imposes substantial energetic demands on female animals, and fertility is often compromised under nutrient limitation. Yet, the neuroendocrine mechanisms linking internal metabolic state to reproductive output remain poorly defined. Here, we identify a population of central Myosuppressin (Ms)-expressing neurons in the brain of *Drosophila melanogaster* that is essential for sustaining fertility during dietary restriction. These Ms+ neurons project to the adipokinetic hormone (AKH)-producing cells (APCs) and undergo activity-dependent synaptic remodeling in response to mating and nutrient cues. Functional manipulation of Ms neuron activity modulates AKH release primarily via Myosuppressin Receptor 2 (MsR2), thereby regulating systemic energy mobilization through AKHR signaling in the fat body. This AKH/AKHR axis promotes nutrient mobilization by inducing the lipase Lip4 and the nutrient transporter Eglp4, enabling reproductive investment under conditions of energetic stress. In addition, the AKH/AKHR axis simulates *yolk protein* expression in the fat body. Together, our findings uncover a brain–APC–fat body neurohormonal circuit that coordinates energy homeostasis and reproductive resilience during nutritional challenge.

O-019 - Neuroendocrine disruption in *Daphnia magna*: linking environmental contaminants to behavior, physiology and emerging disease frameworks

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Understanding how neuroendocrine systems translate environmental cues into changes in physiology and behavior remains a central challenge in comparative endocrinology, especially under complex chemical exposures. In this study, we present Daphnia magna as a useful invertebrate model to examine how environmental neuroactive contaminants interfere with conserved neuroendocrine pathways that regulate behavior, metabolism, reproduction, and development.

We combined newly developed high-throughput behavioral and physiological assays with transcriptomic and metabolomic analyses to capture responses across different biological levels. Using this approach, we found that environmentally relevant chemicals—including the herbicide glyphosate, the antimicrobial triclosan, and selective serotonin reuptake inhibitors (SSRIs)—alter key neuroendocrine systems. In particular, disruptions in dopaminergic and serotonergic signaling were associated with measurable changes in movement, anxiety-like behavior, energy balance, and reproductive performance.

Our findings also point to two emerging themes in invertebrate neuroendocrinology. First, disruption of the microbiota–gut–brain axis in Daphnia underscores the importance of microbiome-driven neurohormonal regulation in shaping behavior and life-history traits. Second, exposure to neuroactive compounds during early life stages leads to lasting physiological and behavioral effects, consistent with the concept of developmental programming and suggesting that these mechanisms are conserved in invertebrates.

In addition, we establish a Parkinsonian-like model in Daphnia. Exposure to dopaminergic neurotoxicants produced characteristic impairments in locomotion and reduced activity within dopaminergic pathways, offering a simple system to investigate neurodegenerative processes from a neuroendocrine perspective.

Finally, we integrate these biological responses into a mixture assessment framework that links chemical exposure to neurohormone-driven behavioral outputs. This approach allows the detection of neuroactive chemical mixtures in environmental samples.

Overall, this work demonstrates the value of invertebrate models for uncovering fundamental aspects of neuroendocrine regulation and its disruption. It highlights connections between ecology, toxicology, and endocrinology, while raising open questions about the evolutionary conservation of neuroendocrine pathways, the role of microbiomes in neurohormonal control, and the long-term effects of early-life exposures on behavior and physiology.

O-020 - The AKH family: invertebrate champions of PTMs

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The adipokinetic hormone (AKH) peptide family is one of the well-researched insect hormone families. The main action of AKHs is the provision of energy during demanding times. Structurally this is also the best investigated invertebrate hormone family. More than 100 bioanalogues have been chemically elucidated. The AKHs have the most post-translational modifications (PTMs) of all known invertebrate hormones. PTM is a process defined as a covalent change to the primary structure of a protein (or peptide) following synthesis and release from the ribosomes. The PTMs of the AKH family are the following:

1. Pyroglutamate formation (pGlu): a glutamine residue of the N-terminus releases ammonia (NH^3), cyclizises and forms 5- oxoproline.

2. Carboxyamidation (-$CONH^2$): the carboxyl group at the C-terminus is converted to an amide group which is provided by glycine.

3. Proline hydroxylation (Hyp): a hydroxyl group is enzymatically added at Cγ position to form 4-hydroxyproline.

4. Thr phosphorylation (pThr): a phosphate group (-$PO^{4\ 3-}$) is reversibly added to the hydroxyl group of Thr.

5. Thr sulfation (sThr): addition of a sulfo group (-SO^3) to the hydroxyl group of Thr.

6. C-Trp mannosylation (Trpglyc): a mannose molecule is added to the C^2 position in the indol ring of Trp.

7. Pro isomerisation: a non-covalent change between trans and cis form of Pro.

These variations beg the question: have the various AKH receptors co-evolved to bind the PTM-AKHs and are such hormones functionally active? This overview answers part of this question.

O-021 - Peptidergic pathways modulating metabolic homeostasis in Drosophila

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Neuropeptides serve as versatile signaling molecules that orchestrate diverse behaviors and physiological responses across changing environmental conditions. While extensive research has demonstrated their critical roles in inter-organ communication and homeostatic regulation, the precise timescales during which neuropeptides coordinate metabolic homeostasis still remain elusive. Due to a lack of genetic tools, a fundamental challenge has been our inability to visualize how peptidergic pathway activity shifts spatially and temporally across different physiological states (e.g. fed versus starved conditions). Exploiting the genetic toolkit of Drosophila melanogaster, we developed Tango-Map MkII sensor, a specialized genetic tool that enables spatio-temporal visualization of neurohormonal signaling dynamics *in vivo.* By integrating Tango- Map MkII with connectomics, single-cell transcriptomics, behavioral genetics, and measurements of multiple physiological parameters, we have systematically characterized peptidergic networks that govern metabolic homeostasis. By mapping when and where neuropeptide signals intersect, we are beginning to understand how neuropeptides fine-tune metabolic homeostasis. This work lays the groundwork for a unifying, testable model of peptidergic communication with implications across multiple homeostatic axes.

O-022 - A role for the neuropeptide myosuppressin and its receptors in male reproductive fitness

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In many animals, including insects, male competition can lead to longer copulation duration, greater ejaculate (sperm and seminal fluid, SF) investment and increased male reproductive success. In Drosophila melanogaster, sperm from the seminal vesicles (SV) is mixed in the anterior ejaculatory duct (aED) with SF from the male accessory glands (MAG). The formation of the ejaculate and the context-dependent modulation of SF investment necessitates tightly coordinated neural and muscular activity. There is, however, a paucity of information on how this is achieved. We have now identified a role for the neuropeptide, myosuppressin (Ms), signaling in the control of sperm and SF transfer to the female D. melanogaster. Ms is expressed in AbG neurons, as well as in an unpaired cell adjacent to the aED and a pair of rectal cells. Ms AbG neurons that innervate the MAG and aED are distinct from the serotonergic AbG (sAbG) neurons that promote ejaculation. Inactivating Ms neurons increased copulation duration and elevated the ejaculate holding period (EHP), while reducing oviposition in the mated female. Results indicate that Ms receptors (MsR1 and MsR2) are expressed in some of the sAbG neurons. Strong expression of MsR2 in the MAG muscle explains the Ms suppression of spontaneous contractions of the MAGs. Loss of a functional MsR2 leads to a 50% reduction of a SF protein in MAGs of virgin males. Taken together, our results suggest that Ms signaling in MAG plays a role in controlling SF investment in the ejaculate, thereby increasing male reproductive fitness.

SOA4 - Endocrine integration of appetite, energy and water balance: lessons from Drosophila

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Energy and water balance are often treated as separate chapters of endocrine physiology. Yet their mechanisms are tightly interconnected. Dehydration not only promotes thirst and water conservation but also affects feeding, metabolic rate, and energy expenditure. Conversely, nutritional state influences osmotic balance and excretory demands. This raises a central question in comparative endocrinology: how do endocrine systems coordinate these interdependent physiological needs? Vertebrate vasopressin illustrates this principle. Beyond its classical anti-diuretic role, vasopressin also acts through extrarenal receptor pathways to influence glucose and lipid metabolism and promote short -termb mobilization of energy reserves. In arthropods, members of the Crustacean Hyperglycemic Hormone (CHH) family provide an evolutionarily distant but conceptually similar solution, with roles in ionic regulation, osmoregulation, and energy metabolism. Here, we focus on Ion transport peptide (ITP), a CHH family hormone in Drosophila melanogaster. We show that ITP acts not merely as an antidiuretic factor, but as a systemic coordinator of thirst, excretion, feeding, metabolic rate, and energy reserve mobilization. Its water-conserving and metabolic functions are partitioned across distinct target tissues and signaling pathways, allowing one endocrine signal to generate multiple physiological outputs. Functions related to water balance are governed by water intake and gut-mediated excretion, with both processes relying on signaling through the membrane guanylate cyclase receptor Gyc76C. The roles of ITP in energy homeostasis involve the regulation of food intake and metabolic rate. ITP also regulates the storage of lipids and carbohydrates. These metabolic functions employ signaling through the tachykinin receptor TkR99D expressed in the fat body, the fly analogue of adipose tissue. Finally, we discuss how ITP signaling interacts with Adipokinetic hormone pathway, revealing a broader neuroendocrine network that coordinates water conservation with energy mobilization. By placing ITP within this broader endocrine network, our work highlights how evolutionarily conserved hormonal systems can couple distinct homeostatic needs, water and energy, through tissue-specific receptor signaling.

O-023 - From intestinal signalling to organismal function: effects of aflatoxin B1 on endocrine–metabolic pathways, feeding and locomotor behaviour in fish

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Aflatoxin B1 (AFB1), a mycotoxin produced mainly by Aspergillus spp., is a frequent contaminant of plant-derived feed ingredients increasingly incorporated into aquaculture diets. Dietary exposure to AFB1 has been associated with impaired growth, tissue integrity and metabolic disturbances in fish. However, little is known about the molecular mechanisms underlying its toxicity, particularly those affecting the intestine, a key endocrine and nutrient-sensing organ involved in the regulation of feeding and energy homeostasis. Moreover, whether disrupting effects of AFB1 translate into alterations in feeding behaviour and locomotor activity remains poorly understood. This study combined complementary *in vitro* and *in vivo* fish models to link the molecular mechanisms of AFB1 intestinal toxicity with its functional consequences at the organismal level.

To characterize the direct intestinal effects of AFB1, rainbow trout *(Oncorhynchus mykiss)* epithelial cell lines derived from the proximal (RTpiMI) and distal (RTdiMI) intestine were exposed to three concentrations (1 nM, 100 nM and 1 µM) of AFB1 in serum-deprived L-15 medium for 2 and 24 h. The relative abundance of genes involved in intracellular calcium signalling, energy sensing and nutrient sensing was quantified by qPCR. Exposure to AFB1 repressed itpr1 and itpr3 (encoding (inositol 1,4,5-trisphosphate receptor types 1 and 3), whereas plcb1 (phospholipase C beta 1) was upregulated in RTpiMI but downregulated in RTdiMI, revealing a segment-dependent disruption of the PLC–IP_3–Ca^{2+} signalling pathway. In parallel, heterogeneous modulation of mtor (mechanistic target of rapamycin) and sirt1 (sirtuin 1) suggested fluctuating metabolic stress rather than a coordinated endocrine–metabolic adaptation. AFB1 produced limited effects on genes involved in fatty acid transporters and sensing, with most significant changes consisting of concentration- and time-dependent modulation of receptor expression.

To evaluate the functional consequences of AFB1 exposure, zebrafish *(Danio rerio)* larvae were exposed to 100 nM and 1 µM AFB1 during early development. Locomotor activity was assessed individually in 4 dpf larvae under alternating light–dark conditions, a widely used proxy for anxiogenic or anxiolytic behavioural responses, using automated video tracking. Feed intake was quantified individually in 10 dpf larvae by measuring gut fluorescence following ingestion of rhodamine-labelled Tetrahymena. AFB1 exposure reduced locomotor activity during both the light and dark phases compared with control larvae, indicating a generalized decrease in locomotor performance, potentially reflecting reduced physiological fitness. Preliminary feeding assays also point to reduced food intake in exposed larvae, suggesting that AFB1 compromises behavioural performance and energy balance at the whole-organism level.

Overall, the present findings identify the intestinal epithelium as an important endocrine–metabolic target of AFB1 and show that exposure to this mycotoxin is associated with altered behavioural responses in fish. The integration of complementary *in vitro* and *in vivo* models provides a multiscale framework to investigate the mechanisms and functional consequences of feedborne mycotoxins.

Acknowledgements: Study supported by Xunta de Galicia and the Natural Sciences and Engineering Research Council of Canada.

O-024 - Orforglipron acts as a pharmacological chaperone to correct cell-surface defective human GLP1R variants

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Our previous work demonstrated that a subset of rare human glucagon-like peptide 1 receptor (GLP1R) variants, linked to increased adiposity and dysregulated glucose control, display markedly reduced cell-surface expression, resulting in impaired GLP1R signaling. Here we discover that orforglipron, a small molecule partial GLP1R agonist, recently approved by the FDA against obesity, acts as a pharmacological chaperone to restore membrane localization and functional activity of most of these GLP1R variants. Orforglipron rescued receptor cell surface expression, recovered cAMP signaling, without inducing receptor internalization, and restored insulin secretion in pancreatic β-cells expressing surface expression defective GLP1R. Notably, orforglipron also enhanced surface expression of the wild-type receptor from human pancreatic β-cells, and potentiated GLP1R-stimulated insulin secretion from human pancreatic β-islets, extending its potential impact. Our findings highlight a therapeutic strategy with a dual function, combining both GLP1R rescue at the plasma membrane with functional activation of GLP1R at the cell surface, offering a potential precision medicine approach for patients with trafficking defects of GLP1R, including GLP1R variant carriers or Wwild-type GLP1R.

O-025 - Ghrelin loss across vertebrate lineages: a consequence of feeding strategies?

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How functionally relevant a hormone module is may be contingent on the ecological context in which it operates. Here, we explore the evolution of ghrelin (GHRL), the 'hunger hormone', a multifunctional peptide regulating appetite, energy balance, and fatty acid metabolism across vertebrates. Its acylation — essential for activity — is catalysed by MBOAT4. By leveraging an extensive set of genomic resources, we report independent inactivation of the GHRL/MBOAT4 module in Serpentes, bird lineages, and selected mammalian lineages, through genomic screening, synteny analysis, and pseudogene inference. Placing these losses in a broader vertebrate context, we find that GHRL dispensability is disproportionately associated with lineages exhibiting intermittent or extreme feeding phenotypes, suggesting a link between dietary specialization and the erosion of appetite-regulatory machinery. More broadly, our results contribute to growing evidence that metabolic hormones are surprisingly prone to secondary loss when dietary shifts erode the physiological contexts in which they operate.

O-026 - Cannabinoids modulate the expression level of genes encoding short neuropeptides F (sNPF) and insulin-like peptides (ILPs) precursors in insects

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Endocannabinoids are naturally occurring lipid signalling molecules that act as the body's internal communication system. Their primary physiological role is to maintain homeostasis by regulating mood, appetite, food intake, pain, and immune response, thereby keep the body in a stable, balanced state. They act as modulators of physiological processes across diverse animal taxa, including mammals, birds, amphibians, fish, sea urchins, leeches, mussels, and even cnidarians. Interestingly, insects do not possess a traditional endocannabinoid system, lacking classic orthologs of cannabinoid receptors (CB1/CB2) and the enzymes required for their degradation. One explanation of this can be lack of endocannabinoid ligands. Insects produce little to no arachidonic acid, which is a precursor in endocannabinoids biosynthesis.

In mammals, modulation of appetite, food intake and metabolism by endocannabinoids occurs, i.a., by interaction with insulin and neuropeptide Y signalisation. We found data that cannabinoids modulate food preference and consumption in *Drosophila melanogaster*. This finding prompted us to check if that results might be mediated by changes in insulin-like peptides and short neuropeptide F signaling. Both pathways play crucial roles in the regulation of metabolism and food consumption in insects.

To address this question, we analysed whether the application of agonists (cannabidiol and anandamide) and antagonist (rimonabant) of cannabinoid receptors affects expression level of genes encoding insulin-like peptides (ILPs) and short neuropeptide F (sNPF) precursors and their receptors (insulin-like peptide receptor (ILPR) and short neuropeptide F receptor (sNPFR)) in Tenebrio molitor larvae.

Our findings indicate that cannabinoids influence expression of analysed genes in brain and ventral nerve cord (VNC). Cannabidiol and anandamide increase the expression level of *ILP1* ad depress *ILP3* and *ILPR*. We did not observe significant changes in expression level of *ILP2* and *sNPF*. The expression of gene encoding of sNPFR was slightly depressed. The effects caused by antagonist – rimonabant – were ambiguous and strongly dose- and tissue-specific.

O-027 - Peripheral FGF21 signalling modulates feed intake and hepatic metabolic pathways in rainbow trout (*Oncorhynchus mykiss*)

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Fibroblast growth factor 21 (FGF21) is a key endocrine regulator of energy homeostasis in mammals, coordinating adaptive responses to fasting, nutrient imbalance and metabolic stress. However, its physiological role in teleost fish remains poorly understood, particularly regarding the integration between feeding regulation and peripheral metabolism. In the present study, we investigated the effects of exogenous Fgf21 administration on feed intake and metabolic regulation in juvenile rainbow trout *(Oncorhynchus mykiss).*

Previous characterization of the endocrine FGF system in rainbow trout revealed a marked divergence from mammalian models, including the absence of detectable hepatic *fgf21* expression under basal conditions and a broad distribution of *fgf21* transcripts in central nervous system regions, intestine and white muscle. In contrast, several fibroblast growth factor receptors *(fgfr)* and *β-klotho* were highly expressed in important peripheral tissues in terms of metabolism, supporting the existence of tissue-specific endocrine signalling pathways. Structural modelling and molecular dynamics simulations further demonstrated stable interactions between Fgf21 and multiple Fgfr isoforms, suggesting a diversified receptor-mediated signalling network.

To evaluate the role of Fgf21 in energy balance regulation, juvenile rainbow trout (60 ± 20 g), obtained from a local fish farm (A Estrada, Spain), were transported to the University of Vigo facilities, where the experimental trial was carried out. Fish were maintained in 100 L tanks under controlled conditions (14 ± 1 ºC; 12 h light:12 h dark photoperiod). Following acclimation, fish were weighed and randomly distributed into experimental tanks. Two complementary experiments were performed. For feed intake evaluation, fish were fed a commercial diet to satiety twice daily and basal feed intake was recorded for 15 days until stable values were reached. Fish were then anaesthetized, weighed and intraperitoneally injected with 500 µL per 100 g body weight of saline solution alone (0.6% NaCl, control) or containing 100 or 200 ng recombinant trout Fgf21 (Fgf21r). Feed intake was recorded at several time points post-administration and expressed relative to previously established basal intake values. In parallel, for metabolic response analyses, fish were subjected to the same administration protocol and tissue samples were collected 2 and 8 h post-administration.

Fgf21r administration significantly affected feed intake, with a significant reduction observed 2 h after administration of the highest dose. In parallel, Fgf21 induced marked changes in circulating metabolites, including increased plasma non- esterified fatty acids (NEFAs) and reduced triacylglycerol levels, indicative of enhanced lipid mobilization. In the hypothalamus, FGF21 also modulated appetite-related neuropeptides and metabolic sensors. These responses support the existence of a coordinated response involved in energy balance regulation.

At hepatic level, Fgf21r appeared to modulate glucose and lipid metabolism together with intracellular signalling pathways. Expression of genes involved in fatty acid uptake and β-oxidation, including *cd36* and *pparα*, increased following treatment, whereas transcripts associated with gluconeogenesis and glucose transport, such as *fbpase* and *glut2*, were downregulated. In addition, Fgf21r activated energy-

sensing pathways through increased *ampkα1* expression together with reduced mtor transcript abundance, supporting a shift towards catabolic regulation.

Overall, the present results demonstrate that Fgf21 acts as an important regulator of energy balance in rainbow trout, integrating feed intake modulation with hepatic metabolic responses and energy-sensing pathways.

O-028 - The role of ITP signaling in the energy metabolism and physiology of *Drosophila melanogaster*

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Introduction: *Drosophila melanogaster*, despite being phylogenetically distinct from Homo sapiens, remains an effective model organism for studying the genetic regulations and pathways governing energy metabolism. While the endocrine regulation of the anabolic pathway via the Insulin signaling is evolutionarily conserved, the hormonal architecture governing catabolic mobilization exhibits significant phylogenetic divergence. In *Drosophila*, the Adipokinetic hormone (AKH) has traditionally been defined as the primary analog of glucagon. However, it does not account for the full spectrum of glucagon's catabolic effects. Recent studies identify Ion Transport Peptide (ITP), a member of the Crustacean Hyperglycemic Hormone (CHH) family, as a critical integrator of energy and water balance. ITP orchestrates physiological homeostasis, maintaining balance between metabolism, excretion, and stress response in *Drosophila*. ITP is also a vital catabolic regulator; it restrains feeding and digestion, accelerates metabolic rate, mobilizes lipid and glycogen stores, and elevates trehalose levels. However, the identity and tissue-specific functions of the ITP receptor(s) in maintaining metabolic and physiological homeostasis remain unclear.

Methods: In this study, we conducted a comprehensive RNAi-based genetic screen of candidate receptors from the literature and identified distinct receptors that partition the physiological outputs of ITP. To understand the role of ITP receptors in physiological regulation, we employed adulthood-specific genetic manipulations primarily using the modified UAS-GAL4 system- the Gene-Switch system to avoid developmental complications. We downregulated the candidate receptors globally and in various tissues using specific GAL4 lines to define the tissue-specific actions of the ITP and understand its role in organismal homeostasis. We measured various physiological and metabolic parameters, like drinking, eating, defecation events, sensitivity to starvation, fecundity, and the levels of stored energy reserves, mainly triacylglycerides, glycogen, glucose, and trehalose. These parameters were measured using established biochemical and physiological assays.

Results and discussion: In *Drosophila melanogaster*, ITP acts as a novel catabolic regulator, regulating various physiological and metabolic parameters. Our findings from ubiquitous candidate receptor knock-down studies indicate the involvement of diverse receptors in mediating ITP phenotypes. Importantly, *GyC76C* is the primary mediator of osmotic regulation, whereas *TkR99D* is essential for energy homeostasis. Through tissue-specific knockdown studies, we highlight that *TkR99D* is required for the mobilization of energy reserves. ITP acts directly via its receptors located on the fat body to initiate the catabolism. The results from this study effectively decouple the metabolic functions of ITP from its roles in excretion. Ultimately, the characterization from the RNAi screen suggests a sophisticated network that highlights the pleiotropic functions of ITP and its significance as a master catabolic regulator of *Drosophila* energy homeostasis, offering a powerful system to understand the complex integration of metabolic and ion balance pathways.

O-029 - Nucleobindin-derived peptides lower lipid levels in hepatocytes

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Nucleobindin-1 (NUCB1) and nucleobindin-2 (NUCB2) are DNA and calcium-binding proteins. Nesfatin-1 (NESF-1), a peptide processed from NUCB2, has been shown to reduce feeding and fat mass, and modulate metabolism. We have identified a nesfatin-1-like-peptide (NLP) processed from NUCB1. NLP shares satiety and metabolic effects elicited by nesfatin-1. In this research, we determined if NESF-1 and NLP regulate lipid accumulation *in vitro*. Human hepatocytes (HepG2/C3A cells) express NLP and NESF-1. Both peptides significantly reduced lipogenic enzyme mRNAs and enhanced beta oxidation enzyme mRNAs in hepatocytes. Lipid contents in oleic acid-induced HepG2/C3A cells were attenuated by 0.1 nM NESF-1 and NLP. Intracellular triglyceride levels were significantly reduced by NES-1 and NLP treatment. This lipid-reducing effect was comparable to that elicited by the positive control (quercetin). The inhibitory effect of NES-1 and NLP on cellular lipid content was blocked by compound C, an inhibitor of AMPK. M30, a short fragment of NESF-1, considered to be its bioactive core, also elicited similar lipid-lowering effects. Transgenic mice lacking Nucb1 or Nucb2 gene exhibited alterations in lipid-regulatory genes, pathways, and processes. The lipid-lowering effects identified here concur with previously published findings from other researchers. It also highlights the potential of nucleobindins and peptides processed from them to address lipid disorders.

TUESDAY, September 8

Plenary Lecture — PL3 (Aula Magna)

Lighting up neuropeptide signaling in context-dependent regulation of behavior

Jan Watteyne, Majdulin Nabil Istiban, Ellen Geens, Vincent Piron, Nisha Kumari, Lara Cardinaels, Isabel Beets, Department of Biology, KU Leuven, Belgium

Neuropeptidergic signaling has important roles in the adaptation of physiology and behavior of all animals. Mounting evidence across species indicates that neuropeptides and their receptors are widely expressed and form an extensive network of extrasynaptic signaling in nervous systems. However, it has been much more challenging to understand how this network is organized, and how it evolves or adapts to regulate context-dependent behaviors. We are investigating these questions in the nematode Caenorhabditis elegans, which has a compact neuroanatomy that is molecularly and anatomically defined at single cell resolution. Using large-scale reverse pharmacology approaches, we mapped the neuropeptidergic signaling network across the C. elegans nervous system. Many peptidergic systems show long-range conservation and are organized in a dense network that has organizational features distinct from the synaptic connectome. Comparative studies across species and life stages shed light on how neuropeptidergic networks adapt to different contexts. In addition, we are developing GPCR-based sensors to visualize context-dependent neuropeptide signaling *in vivo*. We are using these tools to investigate how neuropeptides coordinate homeostatic responses and behavioral adaptations to different internal states. Altogether, these tools shed light on how neuropeptidergic networks are spatiotemporally organized, and how flexible behaviors emerge from network plasticity.

SOA5 - New players, new pathways: emerging mechanisms of gonadotropic regulation of fish spermiogenesis

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The differentiation of haploid spermatids into mature spermatozoa (spermiogenesis) is the least understood phase of fish spermatogenesis despite its critical importance for male fertility. The process involves extensive cellular remodeling of haploid cells, including chromatin condensation, cytoplasmic reduction, mitochondrial reorganization, and flagellum assembly. While early spermatogenesis is known to be regulated by gonadotropins through Sertoli and Leydig cells, the endocrine control of spermiogenesis in fish has only recently begun to be elucidated.

The classical model proposes that follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh) regulate spermiogenesis indirectly by stimulating the production of androgens, particularly 11-ketotestosterone and maturation-inducing progestins. These steroids promote spermatozoa differentiation, whereas Sertoli-cell-derived factors contribute to the local regulation of germ-cell development. However, increasing evidence indicates that gonadotropins exert more direct actions on haploid germ cells than previously recognized.

A major breakthrough was the discovery of luteinizing hormone receptors (Lhcgr) in post-meiotic germ cells of several teleost species. This finding challenged the long-standing view that gonadotropins act exclusively through somatic cells and suggested the existence of endocrine regulation of spermatozoon differentiation from mature spermatid through direct activation of a Lhcgr-mediated cAMP/protein kinase A signaling pathway in these cells, and the induction of genes involved in sperm morphogenesis. The Senegalese sole (Solea senegalensis), which displays semi-cystic spermatogenesis, has emerged as an excellent model to investigate these mechanisms since mature spermatids are released into the seminiferous tubules from Sertoli cell cysts where they complete their differentiation to spermatozoa.

Recent transcriptomic and immunological analyses have revealed that gonadotropins regulate pathways associated with cell adhesion, relaxin and oxytocin signaling, which highlights their role in the disruption of the blood-testis barrier and Sertoli cell-spermatid junctions facilitating spermatid release and Lh paracellular transport into the seminiferous lumen during spermiogenesis. These findings suggest that endocrine control of spermiogenesis extends beyond steroidogenesis to include direct regulation of cellular interactions and tissue remodeling.

Additional complexity has recently arisen from the discovery of two duplicated Lhcgr paralogues, Lhcgrba and Lhcgrbb, in haploid germ cells. Although both receptors are specifically activated by Lh, they exhibit distinct expression dynamics and functions during spermiogenesis. Pharmacological and pathway analyses suggest that Lhcgrba preferentially regulates transcriptional programs involved in chromatin remodeling and the initiation of spermatozoon differentiation, while Lhcgrbb controls pathways associated with cytoskeletal reorganization, flagellum assembly, and sperm functionality. This division of labour reveals an unexpected diversification of gonadotropic signaling in vertebrate germ cells.

Altogether, these new findings support a revised regulatory model of spermiogenesis in which gonadotropins act directly on haploid germ cells in addition to their indirect classical endocrine functions in somatic cells. Understanding how receptor- specific Lh signaling is integrated with androgenic, progestogenic, and Sertoli-cell-derived pathways to coordinate chromatin compaction, sperm morphogenesis, and spermiation represents one of the major challenges in fish reproductive endocrinology.

Acknowledgements:

This work was funded by the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033) and the European Regional Development Fund (ERDF) “A Way of Making Europe” (European Union) through the Grant No. PID2021-126128OB-I00, to F.C., and the Agency for Management of University and Research Grants (Government of Catalonia), Grant no. 2021 SGR 00068 (to J.C.). NL-F was supported by a predoctoral fellowship from the Spanish MCIN (PRE2022-101651). The authors also acknowledge support from the Severo Ochoa Centre of Excellence accreditation (CEX2019-000928-S), funded by the Spanish State Research Agency (AEI; 10.13039/501100011033).

O-030 - CRISPR/Cas9 disruption of srd5a2 reveals essential roles of androgen bioactivation in amphibian reproductive maturation and reproductive success

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Despite its established role in mammals, the physiological importance of Srd5a2 in amphibians remains poorly understood. The enzyme steroid 5α-reductase type 2 (Srd5a2) catalyzes the conversion of testosterone into 5α-dihydrotestosterone (5α- DHT), one of the most potent androgens in vertebrates. Although Srd5a2 is a central regulator of androgen bioactivation in mammals, its *in vivo* role in non-mammalian vertebrates has never been functionally demonstrated. Given increasing environmental pressures from endocrine-disrupting contaminants affecting androgen signaling pathways, understanding androgen metabolism is essential for evaluating reproductive and developmental health in aquatic vertebrates. Here, we generated and characterized the first CRISPR/Cas9 (*srd5a2*$^{+1\Delta4}$) loss-of-function amphibian model in *Xenopus* (*Silurana*) *tropicalis*. Sequencing confirmed a frameshift mutation introducing a premature stop codon predicted to truncate the protein upstream of conserved functional domains. Mutants exhibited reduced *srd5a2* transcript abundance during development and adulthood, consistent with transcript destabilization. Loss of *srd5a2* disrupted multiple androgen-dependent reproductive traits. Homozygous mutant males exhibited impaired courtship vocalizations and reduced amplexus success, leading to altered fertilization outcomes and impaired reproductive performance associated with androgen disruption. Mutant males also displayed severe alterations in androgen-dependent secondary sexual characteristics, including reduced nuptial pad pigmentation, a male trait important for maintaining amplexus behavior. Histological analyses revealed gonadal abnormalities beginning during metamorphic development and persisting into adulthood. Mutants displayed impaired spermatogenesis characterized by empty seminiferous cysts, disrupted cyst organization, delayed germ cell development, and enlarged testes in sexually mature animals. Adult gonads additionally exhibited oocyte-like cells within testicular tissue, indicative of intersex characteristics. To investigate compensatory responses to androgen disruption, expression analyses were conducted on steroid metabolism and androgen signaling pathways during early embryonic development and in adult testes. During early embryonic development, *srd5a1* and *srd5a3* expression remained unchanged, whereas *srd5β* and the *androgen receptor* (*ar*) were upregulated. In contrast, adult mutant testes exhibited increased expression of *srd5a1*, *srd5a3*, and *ar*, whereas *srd5β* expression remained unchanged, suggesting stage-dependent compensatory regulation of androgen signaling and steroid metabolic pathways following *srd5a2* disruption, which reveals previously unrecognized plasticity within amphibian androgen signaling networks. Together, these findings demonstrate that Srd5a2 is required for proper male reproductive maturation, reproductive behavior, gonadal organization, and reproductive success in amphibians. These findings provide a mechanistic framework for understanding how environmental contaminants targeting androgen metabolism may impair reproductive fitness and contribute to amphibian population declines.

Acknowledgments: Marko Horb (National Xenopus Resource, Marine Biological Laboratory)

O-031 - Differential impairment of ventral and dorsal pituitary gonadotroph function by polyendocrine metabolic ovarian syndrome

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Polyendocrine metabolic ovarian syndrome (PMOS), the most common endocrine–metabolic disorder in women of reproductive age, is associated with dysfunctions of the hypothalamic–pituitary–ovarian axis, including an increase in pulsatile secretion of luteinizing hormone (LH). In this context, gonadotropes—specialized endocrine cells—play a key role by responding to gonadotropin-releasing hormone (GnRH) with the pulsatile production and secretion of LH and follicle- stimulating hormone (FSH), thereby regulating the hypothalamic–pituitary–gonadal axis responsible for the control of reproduction in vertebrates. Recent studies have revealed phenotypic and developmental differences between gonadotropes located in the ventral and dorsal regions of the pituitary, suggesting the possibility of a yet uncharacterized functional specialization. In this study, differences in intracellular calcium signaling patterns of gonadotropes from the ventral and dorsal pituitary regions were analyzed in response to different concentrations of GnRH, using pituitaries from male mice, females at different stages of the estrous cycle, and a PMOS model. In addition, LH and FSH secretion was quantified in both pituitary regions across the different experimental groups. Gonadotrope responses were found to vary according to both the stage of the estrous cycle and their pituitary location. In the PMOS model, gonadotropes exhibited increased cellular activity in both pituitary regions. LH secretion was significantly higher in the ventral region compared to the dorsal region, with a marked increase during estrus and in the PMOS model. These findings suggest functional differences in gonadotropes depending on the stage of the cycle as well as their pituitary location, and highlight their involvement in endocrine disorders such as PMOS.

O-032 - Species-specific duplication of gonadal soma-derived factor gene (gsdf) led to sex-biased expression and partial subfunctionalization in European sea bass (*Dicentrarchus labrax*)

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Introduction: In vertebrates, reproduction is regulated by the brain-pituitary-gonad axis through multiple modulators, including gonadotropins, sex steroids, and local gonadal factors, the latter largely unknown in fish. Among these, Gonadal Soma- Derived Factor (Gsdf), a member of the Transforming Growth Factor-β (TGF-β) superfamily, represents a pivotal teleost- specific regulator of reproductive functions, primarily associated with the male sex differentiation. In adult fish, gsdf expression appears restricted to the gonads, although transcript and protein localization and expression timing vary among teleosts. The occurrence of gsdf duplicates in some species, together with the absence of a clearly identified receptor and the structural divergence within the canonical TGF-β cysteine-knot domain, makes this gonadal factor particularly intriguing in a context of still limited knowledge. Based on the identification of two gsdf genes in our previous work, the present study aimed to characterize these paralogues in European sea bass *(Dicentrarchus labrax)*, a key Mediterranean aquaculture species and a well-established model for reproductive biology research.

Materials and methods: A multidisciplinary approach was employed, integrating evolutionary, genomic, and expression analyses at both gene and protein levels. Phylogenetic reconstruction, synteny comparison, and 5' flanking region analysis described gsdf evolution and regulatory conservation among teleosts. Expression of gsdf1 and gsdf2 was evaluated by qPCR in gonads across the lifespan (gonadal differentiation, male puberty onset, annual reproductive cycle). Protein detection and localization were assessed using a specific antibody in gonadal extracts and histological sections. The regulation of gsdf gene expression was evaluated in gonads following *in vitro* and *in vivo* treatments with endocrine and paracrine factors. Finally, transactivation assays in cell lines explored TGF-β receptor signalling pathways that could be stimulated by Gsdf.

Results and Discussion: Phylogenetic and synteny analyses demonstrated that most gsdf duplications are species-specific and not linked to the teleost whole-genome duplication. Promoter analysis revealed conserved transcription factor binding sites (Dmrt1, Nr5a1/2, Sox, Gata), indicating regulatory constraints across teleosts. Gonadal gene expression profiles showed sex-biased patterns: during gonadal differentiation, gsdf1 appeared up to 4-fold higher expressed in males than in females, indicating its participation in testicular differentiation. In male juveniles, both genes were negatively correlated with the onset of puberty. In adults, gsdf1 predominated in males, while in females, gsdf2 expression was up to 10-fold higher than that of gsdf1 in isolated follicular cells, indicating partial subfunctionalization. Gsdf proteins were found in Sertoli cells surrounding SgA in the luminal area of the testis, and in pre-vitellogenic follicular cells in ovaries. Hormonal treatments revealed gsdf responsiveness to E2, Fsh, and Amh in a stage-dependent manner, suggesting integration of gsdf expression within endocrine and paracrine networks during gametogenesis. Transactivation assays in cell lines revealed dynamic type I/ type II receptor pairing and provided experimental evidence for the activation of Smad1/5/8 or Smad2/3 pathways depending on receptor combinations, supporting functional plasticity and redundancy within the TGF-β system.

Conclusion: Collectively, these results suggest that gsdf paralogues have evolved complementary, sex-biased roles in regulating early gonadal development and follicular maturation in European sea bass: gsdf1 is mainly associated with males, and gsdf2 with females. They both participate in gonadal differentiation, may work as early markers of male puberty, and exert a paracrine role in gametogenesis.

Funding: Funded by MCIN/AEI/10.13039/501100011033/ and by ERDF a way of making Europe (PID2021-122929OB-C32). GV supported A.M. PhD contract (GRISOLIAP/2020/129).

O-033 - Polylactic acid impairs endocrine and reproductive function in mammals: protective effects of *Lemna minor* and comparative insights into sperm physiology

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Environmental contamination by plastic-derived materials is increasingly recognized as a major challenge for endocrine and reproductive health. In particular, microplastics (MPs) and nanoplastics (NPs), two pervasive classes of contaminants, are detected in aquatic and terrestrial ecosystems as well as in mammalian and human tissues. Through ingestion, inhalation and dermal exposure, MPs and NPs can cross biological barriers, bioaccumulate and interact with endocrine-sensitive organs including brain and gonads. Little is known about the health impact of biodegradable polymers such as polylactic acid (PLA), considered a sustainable alternative to petrochemical plastics.

Given the continuous increase in exposure to plastic-derived compounds, identifying strategies able to prevent or mitigate their adverse effects has become increasingly relevant. In this context, Lemna minor (LM), an aquatic plant rich in proteins and antioxidant bioactive compounds, represents a promising nutritional protective strategy.

The present study investigated the effects of rhodamine-labelled PLA-NPs on male reproductive physiology, combining *in vivo* exposure in rats with *in vitro* analyses in bovine spermatozoa, and evaluated the potential protective role of LM.

Plasma hormone levels were measured by ELISA. PLA exposure significantly reduced circulating testosterone, estradiol and kisspeptin compared with controls, indicating a clear endocrine-disrupting effect. LM co-administration partially restored all three hormonal parameters. Rhodamine fluorescence analysis revealed PLA accumulation in spermatozoa isolated from both caput and cauda epididymis. Co-localization with prolyl endopeptidase (PREP), a protein involved in sperm function and cytoskeletal organization, showed a pattern consistent with endocrine alterations, with significant changes in the PLA group and partial recovery in PLA+LM animals.

Functional sperm analyses demonstrated a significant reduction in concentration, viability and motility in the PLA group, whereas these parameters improved in PLA+LM animals. Conversely, ELISA analysis of epididymal fluid revealed increased kisspeptin levels in PLA-treated rats, suggesting compartment-specific modulation of the kisspeptin system in response to PLA exposure.

At the molecular level, western blot analyses of testes confirmed extensive PLA-induced dysregulation of pathways involved in reproductive physiology. Steroidogenic markers, including 3β-HSD, CYP19, KISS1, KISS1 receptor and GPR30, were significantly downregulated in PLA-treated animals and partially restored by LM supplementation. The same trend was observed for markers involved in spermatogenic progression and cellular homeostasis, including SIRT1, PCNA and LIN28. In addition,

proteins associated with post-meiotic differentiation and sperm physiology, including HSPA2, MSJ-1, TNP2, AKAP82 and LDH-C, were markedly reduced following PLA exposure and partially recovered in the PLA+LM group.

To further explore the reproductive impact of PLA-NPs and evaluate their direct action on mature gametes, bovine frozen–thawed spermatozoa were exposed *in vitro* to increasing concentrations of rhodamine-labelled PLA-NPs. PLA particles were internalized by spermatozoa, predominantly accumulating in the midpiece, and induced significant modulation of sperm functional responses, including motility-related parameters, mitochondrial metabolism and membrane organization. These observations support a conserved susceptibility of mammalian sperm cells to PLA-derived NPs and strengthen evidence for their reproductive impact across species.

Overall, our findings demonstrate that PLA exposure disrupts endocrine homeostasis, testicular physiology and sperm function, highlighting that biodegradable plastics may also represent a risk factor for male reproductive health. Importantly, LM supplementation significantly attenuated PLA-induced alterations, supporting its protective role against environmentally induced reproductive dysfunction. Together, these results identify LM as a promising nutritional strategy and provide new comparative insights into the reproductive consequences of exposure to biodegradable plastic-derived materials.

O-034 - Brain-derived neurotrophic factor (Bdnf): a regulator of reproduction in zebrafish?

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Brain derived neurotrophic factor (Bdnf) was originally discovered as a brain peptide that aids the growth, repair, and survival of neurons. It is also vital for neuronal plasticity, learning, and memory. In addition, studies, mostly in mammals, have shown that Bdnf is found in several other tissues outside the brain with diverse roles. Bdnf increased oocyte maturation, steroidogenesis, and oocyte developmental competence in different mammalian species. In fish, knowledge of the non-neuronal roles (if any) of Bdnf is limited. Our lab reported that Bdnf increased food intake and regulated energy metabolism in zebrafish. Since successful reproductive events are often tied to positive energy regulation, this research explored the presence and possible action of Bdnf in the zebrafish reproductive axis. First, we characterized the localization of Bdnf and its receptor at gene and protein levels in the tissues of the zebrafish reproductive axis – the brain, liver, and gonads. Next, the action of Bdnf on the expression of hormones, enzymes, and receptors involved in reproduction was assessed *in vivo*. Biological effects on oocyte maturation and vitellogenesis were also tested *in vitro* following incubation of oocytes and liver cells with Bdnf respectively. Then, we studied the intracellular signaling pathways mediating any observed effects of Bdnf in this axis. Finally, using a bdnf mutant zebrafish, the role of endogenous bdnf in reproductive outcomes were assessed. Bdnf and its receptor are present in the zebrafish reproductive axis and enhance the expression of key reproductive genes. Bdnf signals via the Trkb-Mapk/Pi3k/Plc-γ pathways to enhance vitellogenesis in liver cells, and via the Trkb-Mapk/Pi3k to improve oocyte maturation. bdnf-null fish showed reduced response to maturation-inducing hormone and fertilization. Overall, these results confirm a reproductive role, including enhancement of oocyte maturation and fertilization potential, for Bdnf in zebrafish.

O-035 - Temporal redeployment of conserved cell-type programmes underlies seasonal spermatogenesis in European sea bass (*Dicentrarchus labrax*)

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Spermatogenesis is a highly conserved developmental process in which germ cells differentiate within a specialised somatic environment to produce haploid male gametes. In seasonal teleosts such as European sea bass (*Dicentrarchus labrax*), this process is tightly regulated throughout the reproductive cycle, yet lacks a comprehensive, cell-type-resolved, and evolutionarily informed molecular framework.

Here, we combine a histology-anchored bulk RNA sequencing atlas spanning the six canonical stages of testis maturation (Stages I–VI) with publicly available single-cell RNA sequencing datasets from human, cynomolgus macaque, mouse, and zebrafish. Using an orthology-aware comparative approach, we infer conserved spermatogenic cell-type programmes.

Stage-resolved transcriptomics revealed a coordinated temporal regulation of seasonal spermatogenesis. The early stages (SI–SIII) were characterised by spermatogonial maintenance, endocrine signalling, circadian regulation, and the meiotic preparatory machinery. A major transcriptional transition occurred at spermiogenesis (SIV), marked by the activation of flagellar assembly, cytoskeletal remodelling, and extensive chromatin reorganisation. Spermiation (SV) was associated with calcium-dependent signalling, cytokine and chemokine networks, adhesion processes, and extracellular matrix pathways mediating germ-Sertoli cell interactions and sperm release. Testicular regression (SVI) showed strong enrichment of immune activation, endocytosis, phagocytosis, and tissue remodelling, consistent with post-spawning restructuring while preserving the spermatogonial pool.

We identified 132 temporal expression patterns across 9,327 differentially expressed genes and generated three translational resources: (1) stage-informative markers, (2) a curated fertility gene set, and (3) a chromatin-associated compendium.

To resolve these transcriptional dynamics at the cellular level, we constructed a cross-species marker framework by integrating orthologous gene sets from vertebrate single-cell atlases. This analysis identified conserved gene programmes corresponding to spermatogonia (undifferentiated and differentiated), spermatocytes, spermatids, Sertoli cells, Leydig cells, and macrophages. Canonical marker genes (e.g. *fgfr3*, *dmrt1*, *sycp1*, *prm1*, *cldn11*, *cyp11a1*) were conserved across species, supporting preservation of core cell identities.

Importantly, integrating temporal expression and cell-type assignment revealed that seasonal spermatogenesis is driven by temporal redeployment of conserved transcriptional programmes, rather than extensive rewiring of cellular identity. Early stages were dominated by conserved chromatin and epigenetic programmes linked to stem cell maintenance and meiotic competence, whereas later stages displayed increasingly lineage-specific transcriptional signatures associated with sperm function, immune-mediated tissue remodelling, and reproductive strategy.

Methodologically, we demonstrate that strict orthology-based integration can be extended through annotation-aware and orthogroup-based inference approaches to maximise gene recovery while

maintaining biological interpretability, enabling robust cell-type inference in non-model organisms lacking single-cell data.

Collectively, this work establishes the first integrated, cell-type-informed, and evolutionarily grounded transcriptomic framework for seasonal spermatogenesis in a marine teleost. Beyond advancing fundamental understanding of vertebrate reproduction, it provides stage-specific markers and candidate regulatory pathways with applications in aquaculture, including the control of maturation timing and fertility in European sea bass.

Acknowledgements: supported by the Spanish Ministry of Science and Innovation through project PID2021-122929OB-C31 to MB. and an FPI doctoral fellowship (PRE2021-099652) to NS. N.D. was supported by a Severo Ochoa postdoctoral fellowship (CEX2019-000928-S) and a Marie Sklodowska-Curie Postdoctoral Fellowship (HORIZON-MSCA-2021-PF-01; grant number 101066799).

O-036 - Transposable element evolutionary age shapes DNA methylation dynamics during spermatogenesis in European sea bass (*Dicentrarchus labrax*)

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The European sea bass (*Dicentrarchus labrax*) is an economically important marine teleost with a seasonal reproductive cycle in which spermatogenesis proceeds through six successive canonical stages of gonad maturation (SI-SVI). This process is hormonally regulated via the brain-pituitary-gonad (BPG) axis. The brain acts as the key organ of the BPG and controls the expression of gonadotropins and androgens involved in gonadal development. While stage-specific gene expression has been extensively characterised, the epigenetic and regulatory landscape underlying spermatogenesis remains poorly understood. Transcriptional regulation is often mediated by transposable elements (TEs), small DNA fragments that can spontaneously change positions within the genome, whose activity and regulatory potential are tightly controlled by DNA methylation and evolutionary history.

Here, we investigate DNA methylation dynamics across spermatogenesis and assess the influence of TE evolutionary history on epigenetic regulation. We show that spermatogenesis is accompanied by large-scale, stage-dependent reorganisation of the methylome, with progressive divergence from the immature stage (SI), peaking at mid- to late stages (SIII-SV), and decreasing during the recrudescent stage (SVI). These changes predominantly affect intragenic and distal regulatory regions rather than canonical promoters.

We identify TEs as a major substrate of spermatogenic methylation dynamics. Young TEs undergo marked hypermethylation during active spermatogenesis (SIII-SV), consistent with targeted repression and genome protection during germ cell development, whereas older TEs tend to exhibit lower methylation levels, consistent with a progressive relaxation of methylation constraints with evolutionary age. In contrast, the recrudescent stage (SVI) shows increased variability and reduced methylation levels, suggesting a relaxation of epigenetic control. Methylation patterns also differ by TE class, with some families being preferentially hypermethylated (e.g., LINEs: long interspersed nuclear elements), while others remain relatively hypomethylated (e.g., LTRs: long terminal repeats), revealing class-specific regulatory dynamics. For the latter, a non-linear effect is observed, with a pronounced methylation shift at intermediate evolutionary ages that stabilises in older TEs. A subset of hypomethylated TEs does not follow canonical patterns, suggesting additional layers of regulation, potentially linked to chromatin state, cellular composition, or stage transitions.

Integration with gene expression data revealed a moderate but non-random association between DNA methylation and transcription, with the strongest coupling observed at promoter-associated and TE-linked regulatory regions.

Together, these results show that endocrine-regulated spermatogenesis is accompanied by coordinated, genome-wide epigenetic reprogramming shaped by the evolutionary context of TEs. This work provides new insights into endocrine regulation of spermatogenesis in fish and highlights the role of epigenetic mechanisms in seasonal reproduction.

Acknowledgements: supported by the Spanish Ministry of Science and Innovation through project PID2021-122929OB-C31 to MB. and a student contract to NP. N.D. was supported by a Severo Ochoa postdoctoral fellowship (CEX2019-000928-S) and a Marie Sklodowska-Curie Postdoctoral Fellowship (HORIZON-MSCA-2021-PF-01; grant number 101066799).

O-037 - Evolutionary and functional insights into CB1 signaling in spermatozoa: implications for endocrine control of reproduction

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Gametogenesis is a complex process requiring precise regulation by the hypothalamic–pituitary–gonadal axis. In addition to classical hormonal pathways, reproductive function is also modulated by lipid-derived bioregulators collectively known as endocannabinoids. Together with their receptors and metabolic enzymes, these molecules constitute the endocannabinoid system (ECS), an evolutionarily conserved signaling network recognized as part of the endocrine regulation of reproduction. Among ECS components, cannabinoid receptor 1 (CB1) is implicated in the control of sperm maturation, motility, and capacitation.

Nevertheless, the localization and function of CB1 in human sperm remain controversial, largely due to limitations of previous widefield microscopy studies. Using confocal and Airyscan microscopy, we mapped CB1 distribution in human sperm and identified a dotted pattern along the flagellum, presence in subsets of midpieces, and discrete labeling in the sperm head. Comparative analyses further revealed that CB1 localization in the sperm tail is conserved across vertebrate and invertebrate species, whereas head localization was restricted to roosters, where it was confined to the acrosomal region, and mammals. In mammalian sperm, a subset of CB1 receptors was additionally detected intracellularly, beneath the plasma and outer acrosomal membranes and extending toward the nuclear region, where labeling persisted after the acrosome reaction.

These observations support functions for CB1 beyond the established roles in sperm motility and capacitation. Since CB1 signaling has been associated with chromatin remodeling in murine sperm, we investigated whether a similar mechanism exists in human spermatozoa. Activation of CB1 with the selective agonist Arachidonyl-2'-chloroethylamide (ACEA) significantly increased histone H4 acetylation and restored acetylation levels in human asthenoteratozoospermic samples to values comparable to normozoospermic donors. In human asthenoteratozoospermic samples, treatment with N- arachidonoylethanolamine (AEA) improved DNA integrity by reducing sperm DNA fragmentation; however, the inability of ACEA to reproduce this effect indicates that other receptors beyond CB1 contribute to this protective mechanism. Since the histone-to-protamine transition represents a key step in mammalian chromatin remodeling, our findings support a conserved role for CB1 in regulating chromatin dynamics during mammalian sperm maturation and highlight the ECS as an important endocrine modulator of male reproductive function.

Acknowledgements:

This study was funded by a seed microproject “FertConcern” (EURECA-PRO 20, Universidad de León) and Ricerca Scientifica Ateneo 2022 to O.C. M. Lombó is supported by the programme MSCA Postdoctoral Fellowships 2024 (Horizon Europe, project number 101209183).

SOA6 - Mineralocorticoid receptor activation and stress-related behaviour: insights from single cell sequencing of zebrafish brain

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The mineralocorticoid receptor (MR) regulates stress-related physiology and behaviour in vertebrates, and the primary hormone mediating these effects is aldosterone. Teleosts lack aldosterone, leading to the proposal that cortisol is the primary ligand for MR activation in fish. Studies have proposed that 11-deoxycorticosterone (DOC) may be a potent ligand for MR activation in fish, but this has been confirmed only by *in vitro* reporter assays. To date, no studies have established DOC as a bona fide physiological ligand for MR activation in fish. We purified the ligand-binding domain (LBD) of zebrafish MR and determined the crystal structure of the MR LBD with DOC to 1.85Å. The results showed that the zebrafish MR LBD binding pocket is highly conserved. Isothermal titration calorimetry is being carried out to establish the binding affinities of various corticosteroid ligands to the purified zebrafish MR LBD. When zebrafish larvae were acutely stressed, larval DOC levels increased at 10 min but dropped to resting levels at 30 min post-stressor, similar to the cortisol response. DOC exposure to larval zebrafish led to a hypoactive behavioural phenotype, a response distinct from glucocorticoid receptor (GR) activation, which causes hyperactivity. The DOC-mediated behavioural response was abolished by the MR antagonist eplerenone and in the MR knockout larvae, confirming MR-dependent signalling. This was further confirmed by rescuing the behavioural and transcriptional responses to DOC by reintroducing MR into the knockout embryos. Co-treatment with cortisol abolished the DOC-induced hypoactivity, suggesting that elevated cortisol levels modulate DOC-MR signalling. To determine the brain regions and cell-specific transcript responses to stress associated with MR activation, single-cell sequencing of the adult zebrafish brain after cortisol treatment was carried out in the wild-type and GR and MR knockout mutants. Single-cell transcriptome analysis revealed distinct MR and GR transcript abundance in neuronal and nonneuronal populations, which were differentially altered by elevated cortisol levels. The talk will highlight how these changes affect stress signalling, providing insights into MR's role in shaping the stress-related behavioural phenotypes in zebrafish.

O-038 - Mineralocorticoid and glucocorticoid receptor heterodimers drive distinct cortisol-induced stress responses

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The steroid hormone cortisol plays a central role in the physiological response to stress, influencing multiple systems, including the brain and immune system. These effects are mediated through two receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Both receptors act as ligand-activated transcription factors that bind as homodimers to response elements in target genes. Although MR and GR are also capable of forming heterodimers, the physiological significance of MR/GR heterodimerization has remained largely unclear. In recent studies, we demonstrated that MR/GR heterodimerization is required for specific cortisol-induced behavioural responses, particularly the hyperactivity observed in the light/dark assay. We generated mutant forms of MR and GR that selectively form homo- or heterodimers and we expressed these receptors in zebrafish larvae from an MR/GR double knockout line. Behavioral analysis of these larvae revealed that cortisol-induced hyperactivity occurred exclusively when MR/GR heterodimers could form. To identify downstream molecular targets of the MR/GR heterodimers, we subsequently performed RNA sequencing. The results showed that MR/GR heterodimers regulate distinct transcriptional programs, including genes involved in glutamatergic signalling. This was further validated through CRISPR/Cas9-mediated editing of a response element in the gene encoding the metabotropic glutamate receptor 3, which abolished the cortisol-induced hyperactivity phenotype. Interestingly, we also found that certain anti-inflammatory actions of cortisol depend on MR/GR heterodimerization. Specifically, cortisol-induced inhibition of macrophage migration required the formation of MR/GR heterodimers. Together, our findings establish the physiological relevance of MR/GR heterodimerization *in vivo* and highlight its key role in mediating cortisol-driven stress responses at both behavioural and immune levels.

O-039 - Tissue-specific regulation of cortisol clearance by 11βHSD2 in rainbow trout

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The endocrine stress response coordinates cortisol production to help vertebrates cope with environmental and physiological challenges. As the primary glucocorticoid in teleost fish, cortisol plays a critical role in maintaining homeostasis during stress. While transient elevations in cortisol are adaptive, prolonged exposure can impair processes such as growth, reproduction, immune function, and neurogenesis. Consequently, mechanisms that terminate cortisol signalling and restore baseline corticosteroid levels are essential for physiological resilience. However, compared to cortisol synthesis, the mechanisms underlying cortisol clearance remain poorly understood despite their likely importance during stressor recovery.

In fish, the conversion of cortisol to its inactive metabolite, cortisone, is irreversible and mediated by 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). Therefore, 11βHSD2 may regulate both local cortisol signalling and circulating corticosteroid levels during recovery from stress. To better understand the physiological role of 11βHSD2, we profiled circulating and tissue levels of cortisol and cortisone in rainbow trout during the hours following an acute stressor. We also investigated how inhibiting 11βHSD2 affects baseline and post-stressor corticosteroid dynamics.

After an acute stressor, circulating cortisol peaked earlier and declined more quickly than cortisone, suggesting that rapid cortisol clearance is prioritized during recovery. In tissues, both cortisol and cortisone content increased post-stressor, but dynamics varied across tissues. Some tissues (e.g., brain and gill) maintained their cortisol-to-cortisone ratio post-stressor, suggesting a higher capacity for cortisol conversion. In contrast, other tissues (e.g., muscle and spleen) showed significant increases in this ratio, suggesting a lower capacity for cortisol conversion. Supporting a key role for 11βHSD2, higher baseline transcript abundance of 11βhsd2 was associated with a better ability to maintain tissue cortisol-to-cortisone ratios post-stressor. Pharmacological inhibition of 11βHSD2 activity significantly elevated baseline cortisol levels and attenuated the decline in plasma cortisol observed during recovery, although it did not prevent the initial stress-induced rise in cortisol.

Although multiple routes for cortisol clearance exist, these findings identify 11βHSD2 as a tissue-specific and essential regulator of cortisol dynamics in fish. By limiting tissue exposure to cortisol during recovery, cortisol metabolism may help preserve the adaptive benefits of acute stress responses while protecting against the detrimental effects of prolonged glucocorticoid elevation.

O-040 - Gut-derived tachykinins regulate osmotic and nutritional homeostasis in *Drosophila melanogaster*

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Neuroendocrine systems are complex signaling networks that are critical for regulating vital physiological processes including metabolism, excretion, and reproduction. In the fruit fly, *Drosophila* tachykinins (DTKs) are neuropeptides produced in brain neurons and in midgut enteroendocrine cells (EECs), which may function as circulating hormones. Our work recently established the diuretic role of DTKs in regulating ion-water balance in the adult fruit fly by targeting the DTK receptor (DTKR) expressed in stellate cells of the Malpighian 'renal' tubules (MTs). However, whether EECs are the source of DTK hormones targeting peripheral organs like the MTs to induce diuresis is currently unknown. Therefore, the current study aimed to investigate the role of gut-derived DTKs following osmotic and nutritional stress in the adult fruit fly. To investigate this objective, RNA interference was utilized to knock down DTK expression in gut EECs to assess the functional impact of DTK in whole animal bioassays involving environmental and nutritional stresses. Additionally, DTK abundance was quantified in gut EECs of control flies following various stress and diet conditions. Our data reveals that DTK knockdown increased survival following desiccation and ionic stress and flies retained higher water content levels compared to control flies. We next examined how DTK levels changed in flies following various stress and diet conditions, revealing significantly higher DTK immunoreactivity in gut EECs following either ionic or starvation stress, and in flies fed a high-carb diet. Comparatively, desiccation stress, along with flies fed a high-fat or high-protein diet, resulted in lower DTK immunoreactive staining. Overall, this research provides further insight into the role of DTKs in osmotic and nutritional balance, and ongoing work aims to determine ion and metabolite levels in circulation following stress conditions under normal and disrupted DTK-DTKR signaling.

O-041 - Hypothalamic-pituitary interrenal axis activation in embryonic fishes. Challenging the claim that the axis is hyporesponsive

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Anthropogenic activities are increasingly changing our global climate. In addition to chemical pollutants, fishes are facing a rise in water temperatures and hypoxic episodes coupled with greater variability and extremes. Early life stages are particularly vulnerable to environmental changes and stress and are a potential bottleneck to recruitment and population survival. My research group has been studying the short- and long-term effects of thermal and hypoxic stress on embryos and larvae of cold and cool freshwater fishes collected from the wild . Recent work has focused on the ontogeny and activation of the hypothalamic-pituitary axis in embryos of Lake Whitefish and Walleye which are important economic, social, and cultural fishes in Canada and the Northern United States. Evidence clearly supports the importance of glucocorticoids (i.e., cortisol or corticosterone) in embryonic and post-embryonic development of vertebrates from fishes to humans. Fish embryos rely largely on maternally deposited cortisol for embryonic development and increasing or decreasing ovum cortisol levels will often disrupt embryogenesis. There is a general acceptance in the field that the hypothalamic-pituitary (HPI) axis in fish embryos is immature and hyporesponsive until some point after hatch. This notion is derived from the inability to activate the HPI-axis or cortisol synthesis until this time; however, the absence of a biological response to a treatment or stressor does not provide strong or conclusive evidence that it is nonexistent. In this presentation I will challenge the idea that the hypothalamic-pituitary axis of all fish embryos is hyporesponsive. Work in my laboratory showed that whole embryo transcript levels of *crh, crhbp, pomc* and *star*, and cortisol increase in response to hypoxia in Lake Whitefish embryos at 38 dpf (~32% developed); at this stage the heart is developing and nearing its first heartbeat and is therefore a sensitive, critical embryonic stage (Whitehouse et al., Gen Comp Endocrinol 295:113524). In a recent follow-up study, we provide evidence that handling and hypoxia stress can upregulate transcript levels of *crh, pomc, cyp11a1* and *star2* in Walleye embryos at 5 (~18 % developed; gastrulation) and 11 dpf (39% developed; heart development). Data throughout embryogenesis and post-hatch will be presented. Ongoing work is quantifying both transcript levels of glucocorticoid receptors 1 and 2 and the mineralocorticoid receptor, and whole-body cortisol levels in these Walleye embryos and larvae. Collectively, our research shows that some fish embryos can activate the HPI-axis and synthesize cortisol de novo in response to stress. One key difference between the two studies conducted in my lab and previous studies is the frequency of sampling in the early embryonic phase. I hypothesize that although fish embryos likely have a period where the HPI-axis is hyporesponsive, this is not characteristic of either the entire embryonic period or all fishes. Activation of the HPI-axis during critical windows of development such as heart formation works in conjunction with cellular stress responses and serves as an important protective function in fish embryos when exposed to stress.

O-042 - Acute stress enhances macrophage responsivity during inflammation in zebrafish

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It is well established that stress and the attendant rise in glucocorticoids (GCs) results in immune suppression. However, recent evidence suggests that acute stressors can augment innate and adaptive immune responses, altering both the location and responsivity of leukocytes. Leukocytes, such as macrophages, are highly responsive to GCs due to the expression of the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) in these cells, yet little is known regarding the immunological significance of acute stress on macrophage distribution. Here, we tested the hypothesis that upon acute stress, macrophages would relocate to the periphery to increase barrier immunity. To study this, we used transgenic zebrafish harbouring fluorescently tagged macrophages, allowing for real-time visualization of the immune response to stress. We first observed that macrophages rapidly redistributed towards the dermis post-stress and that this was dependent on both GR and MR's modulation of the macrophage CXC chemokine receptor type 4 (Cxcr4). Indeed, mutagenesis of a key glucocorticoid response element in the promoter region of cxcr4a completely abolished stress-induced macrophage redistribution. We further determined that macrophage migration towards the periphery was promoted by melanocyte production of a Cxcr4 ligand (cxcl12a). Additionally, another subset of macrophages was also highly responsive to an inflammatory stimulus, with nearly double the amount of macrophages migrating towards a wound site. Responsivity of macrophages at the wound site was also characterized by a distinct molecular mechanism, as macrophage migration depended on the Cxcr3/Cxcl11 axis. Finally, to confirm the immunological significance, we added a fluorescent, soluble antigen and observed a marked increase in antigen uptake by macrophages of acutely stressed larvae. Taken together, our data suggest immunity in barrier tissues can be increased under conditions of acute stress and that this may be part of a larger programme of stress-induced immune enhancement.

O-043 - Modulation of HPI axis and GH/IGF system through a dietary *Cannabis sativa*-based additive to improve gilthead seabream (*Sparus aurata*) resilience under chronic stress

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In intensive aquaculture, high stocking density represents a chronic stressor that can compromise fish welfare and affect growth. Due to the growing concern for animal welfare and sustainability in aquaculture, dietary supplementation with functional additives is proposed as an alternative to improve the stress response triggered by stocking density. This study evaluated the efficacy of a dietary phytogenic additive (TecnoGMP), based on hemp (*Cannabis sativa*) seed oil, lemon balm (*Melissa officinalis*) and passionflower (*Passiflora incarnata*), in mitigating the negative impacts of high stocking density on gilthead seabream (*Sparus aurata*) welfare status. Animals were randomly distributed into 12 tanks within a RAS, establishing four different rearing conditions in triplicate based on the combination of aquafeed (control or supplemented with 0.15 % TecnoGMP), and stocking density (low initial stocking density, LSD: 8.5 kg/m^3; or high initial stocking density, HSD: 42.0 kg/m^3). After 70 days, the animals were euthanized and brain, pituitary, head kidney (HK) and liver samples were collected to evaluate changes in target genes involved in the Hypothalamic-Pituitary-Interrenal (HPI) axis and GH/IGF system regulation. Additionally, plasma samples were obtained to assess cortisol levels, as a primary stress response.

The results suggest that aquafeed supplemented with TecnoGMP exerts a relaxing effect by attenuating HPI axis activation at the central level induced by HSD condition. In the brain, this diet induced a downregulation of *crh* and *crhbp*, as well as serotonergic receptor (*htr1aa*) expression, regardless of the diet, indicating an inhibition in the stress cascade. The expression of brain *hsd11b*, which is related to cortisol deactivation, decreased due to the high density. Conversely, in the HK, a reduction in *star* and *cyp11b* expression was observed in fish maintained at this density and fed the control diet, suggesting an allostatic overload of the HPI axis. However, TecnoGMP diet decreased pomca expression in the pituitary, regardless of the stocking density, and maintained *star* and *cyp11b* expression at HSD similar to LSD. Regarding growth regulation, HSD enhanced brain *ghrh* expression, although this did not translate into changes in pituitary *gh* expression. However, pituitary *ghr* expression enhanced at this density, suggesting the promotion of GH physiological actions, like the stimulation of catabolism to meet energy demands under this stressful situation. The aquafeed supplemented with TecnoGMP additive modulated the effect of HSD, leading to a decrease in ghra and ghrb expression in the pituitary. Hepatic *igf1* expression, and that of the receptors *igf1ra*, and *igf1rb*, were unaffected by neither diet nor density, but TecnoGMP appeared to upregulate *igf1* expression in the muscle, though it was not statistically significant. These findings demonstrate the potential of TecnoGMP to exert a relaxing and a growth-modulating effect by attenuating HPI axis activation induced by HSD at a central level. Additionally, the results highlight the potential of this phytogenic supplementation as a nutritional strategy to enhance gilthead seabream welfare and physiological resilience under intensive aquaculture conditions.

O-044 - When the hive gets high: Cocaine's effects on honeybee biology and society

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Cocaine addiction poses a significant global challenge with far-reaching medical, economic, social, and legal consequences. Advancing our understanding of cocaine's mechanisms of action is therefore of critical importance. Because ethical and practical considerations preclude many experimental approaches in humans, animal models are indispensable. Despite the predominance of mammalian models in addiction research, invertebrates remain rather underexplored. Here, we demonstrate the utility of the honeybee (*Apis mellifera*) as a complementary model for studying the effects of a single cocaine exposure, spanning responses from the individual organism to the social organization of the colony.

At the individual level, a single cocaine dose triggered a biphasic response comprising two temporally separated axes. The early “neurochemical–behavioural” axis involved changes in biogenic amine levels that recapitulated hallmark neurochemical responses observed in mammals. In contrast, the later “subcellular–toxic” axis was characterized by mitochondrial dysfunction, reduced citrate synthase activity, and activation of antioxidant defence mechanisms, revealing the emergence of cellular stress and metabolic dysregulation.

To evaluate colony-level consequences, vibroacoustic communication was monitored following cocaine exposure. Alterations in these signals emerged with a marked delay relative to the early biochemical and ultrastructural changes observed in individual bees, suggesting that collective responses arise only after the disruption of cellular and metabolic homeostasis has propagated to the social level.

Taken together, our results highlight the honeybee as a powerful complementary model for cocaine research, offering a unique opportunity to investigate cocaine-induced effects across multiple levels of biological organization, from molecules and cells to behaviour and social interactions. By bridging these scales, this approach yields new insights into the systemic actions of cocaine and contributes to a more comprehensive understanding of addiction-related processes.

Supported by grants No. 24-10662S (GAČR) and No. LUAUS25142 (MŠMT).

Plenary Lecture — PL4 (Aula Magna)

Wiring physiology: neuroendocrine control of insect homeostasis through organ crosstalk

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Animal homeostasis depends on two complementary strategies: functional specialization of individual organs and endocrine coordination between them. Yet understanding how molecular and cellular signals are organized into dynamic, whole-animal responses to environmental change remains a dauting challenge. In this talk, I will show how genetically tractable insect models offer distinct advantages to investigate both levels of regulation. In keeping with the August Krogh principle, insect models combine experimental flexibility with powerful genetic tools, enabling endocrine mechanisms to be studied from molecules and cells to organ function and whole-animal physiology in ways that remain difficult in conventional models. To illustrate this point, I will first discuss how studies in the red flour beetle Tribolium castanuem challenge the classic two-cell- type model of insect renal physiology. By integrating cell and tissue-specific transcriptomics with advanced bioimaging and physiological analysis, our work has revealed that both the organization of the renal epithelium and the division of transport functions into distinct cell types differ fundamentally in beetles. These findings illustrate how evolutionary changes in cellular architecture can generate distinct physiological solutions to the universal challenge of maintaining water and ion balance. Further, since water conservation strongly constrains survival, such renal innovations may have contributed to the extraordinary ecological diversification of beetles. I will then turn to the fruit fly Drosophila melanogaster to examine how homeostasis is coordinated across the whole animal. Our recent work identifies gut enteroendocrine cells as peripheral osmosensors that detect ingestion-related hypotonicity, in which cell swelling and membrane stretch trigger Ca^{2+} influx and release of Diuretic Hormone 31 (DH31) into the circulation, in a manner that is analogous to that observed for vasopressin secretion in mammals. Rather than acting on a single target organ, DH31 signaling recruits neural, renal and intestinal effectors to coordinate drinking behaviour, renal fluid secretion and gut motility. In sum, this gut–brain–renal axis thus converts a local sensory event into an anticipatory, organism-wide response that serves to buffer harmful osmotic disturbances. These findings raise the possibility that ingestion-coupled osmotic sensing by the gut represents a more general physiological principle, through which endocrine signals anticipate and limit disturbances before they manifest systemically. Together, these studies demonstrate how homeostasis emerges from the interplay between cellular specialization, organ function and neuroendocrine communication, and highlight the value of comparative insect models for uncovering universal principles of endocrine regulation that may extend across animal systems.

SOA7A - Evolutionary and functional crosstalks between the thyrotropic and gonadotropic axes

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The thyrotropic and gonadotropic neuroendocrine axes play a crucial role in controlling key stages of vertebrate life cycles such as development, metamorphosis, migration, puberty and reproduction. The lecture will review some of the close relationships between these axes from the perspectives of molecular evolution and functional interactions.

At the brain level, the key-player of the gonadotropic axis is the decapeptide gonadotropin-releasing hormone (GnRH), while the tripeptide thyrotropin-releasing hormone (TRH) and/or the polypeptide corticotropin-releasing hormone (CRH) are involved in the thyrotropic axis. As CRH is primarily known for its role in the corticotropic axis, CRH may coordinately activate both corticotropic and thyrotropic axes. CRH also links various regulatory functions via the ultimate production of corticosteroids, which interact with thyroid hormones and sex steroids on various target tissues.

The main molecular homologies between the thyrotropic and gonadotropic axes concern pituitary glycoprotein hormones. Thyroid-stimulating hormone (TSH) and gonadotropins (LH and FSH) are all composed of homologous alpha and beta subunits, which share a common evolutionary history with the ancestral glycoproteins GPA and GPB. Likewise, TSH, LH and FSH membrane G protein coupled receptors (GPCR), expressed by the thyroid and the gonads, respectively, are also homologous. Diversification of glycoprotein hormone subunits and receptors occurred mainly via vertebrate whole genome duplications. Likely related to this common evolutionary history, crosstalks may be supported by some cross-reactions between hormones and receptors, cross-expression in target glands and tissues, and involvement in common regulatory networks. Striking examples are provided by the gonadal expression of TSH receptor, or by the action of TSH at the brain level for the triggering of seasonal reproduction.

Thyroid hormones and sex steroids are produced by the thyroid and the gonads, under the regulation by TSH and LH/FSH, respectively. Local production and metabolism of thyroid hormones and sex steroids also take place in various other tissues including brain, pituitary and peripheral target tissues. Thyroid hormones and sex steroids, as well as corticosteroids, mainly act via receptors that belong to the large nuclear receptor superfamily likely reflecting a long-term common ancestral origin. Via their nuclear receptors, thyroid hormones and sex steroids act directly at the DNA level on target genes, which confers to these hormones remarkable morphogenetic properties as shown during development, metamorphosis or puberty. Multiple interplay between thyroid and sex steroids hormones are supported by shared target tissues and target genes.

The shared evolutionary histories and multiple cross-talks of the thyrotropic and gonadotropic neuroendocrine axes open new research perspectives on their roles in the evolution and adaptation of vertebrate life cycles.

SOA7B - Thyroid hormone action in neurodevelopment. A surprisingly early kickoff

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The thyroid hormone (TH) T3 is a well-established and critical orchestrator of various central nervous system (CNS) developmental events. This hormone acts as a timing signal that coordinates and aligns the parallel development of different neuronal systems and associated glial cells; temporally, the requirement for T3 varies with developmental stage. Until now, it is well accepted that the onset of TH action occurs after neural tube closure, after neural progenitor cells (NPCs), a multipotent population capable of generating both neuronal and glial lineages, are present. At this developmental stage, TH action has mainly been associated with oligodendrocyte precursor cell (OPC) differentiation into mature oligodendrocytes (OL); these are myelin-producing glial cells of the CNS and arise from NPCs through a process tightly regulated by multiple molecular cues, including T3. However, the molecular mechanisms driven by T3 that underlie these processes remain unclear.

We investigated the effects of discrete changes in T3 bioavailability in the NPC-to-OPC transition, which in the zebrafish occurs at around 30 hours post-fertilization (hpf). Three experimental paradigms were used: immersion of embryos in a medium supplemented with a single T3 load for 20 h and generation of dio2 and dio3b gene crisprants, in which intracellular T3 activation or inactivation, respectively, is impaired.

We observed that NPC and OPC markers were downregulated in the brains of overexposed larvae (qPCR) in a time- and region-specific manner. Whole-mount immunostaining revealed reduced expression of OPC markers and reduced myelin content in the brains of T3-treated larvae, suggesting a reduced OL population. This was not evident in crispants. Together, these findings suggest that early T3 time-specific fluctuations may impair NPC proliferation or differentiation towards the oligodendroglial lineage. To assess whether this was due to a shift in NPC differentiation towards the neuronal lineage, we analyzed neuroblasts and mature neurons in larvae and observed increases in both populations across the three experimental paradigms. These findings suggest that T3 may play a role in neural cell fate at earlier stages of neurodevelopment and further emphasize that the timing, duration, and spatial distribution of TH signaling are critical determinants of neurodevelopmental outcomes.

Acknowledgments: This study was supported by Grants from PAPIIT IN223026-3 and IN206724. We thank the technical support of Ramón Martínez Olvera and Mariana de la Teja Peñaloza.

O-045 - Molecular effects of thyroid hormone and histone methyltransferase Dot1L during *Xenopus tropicalis* metamorphosis

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Epigenetic regulation is central to vertebrate development, with histone modifications governing stage and tissue-specific gene expression programs. Methylation of histone H3 at lysine 79 (H3K79) is catalyzed exclusively by the evolutionarily conserved methyltransferase Disruptor of Telomeric Silencing-1-like (Dot1L), whose dysregulation drives multiple cancers, including pediatric leukemias. Nevertheless, the physiological functions of Dot1L during normal vertebrate development remain poorly understood. Using Xenopus tropicalis as a tractable model of post-embryonic development, we previously identified Dot1L as both a direct transcriptional target and a coactivator of the thyroid hormone receptor (TR), which mediates the actions of triiodothyronine (T3) during metamorphosis. Dot1L knockdown severely impaired tadpole development, causing lethality before metamorphic onset. To define the *in vivo* requirement for Dot1L catalytic activity, we generated TALEN-mediated mutants carrying an in-frame 18 bp deletion within the methyltransferase domain. Homozygous mutants exhibited complete loss of H3K79 methylation, confirming Dot1L as the unique H3K79 methyltransferase in X. tropicalis, and phenocopied the knockdown lethality, establishing that enzymatic activity is essential for early post- embryonic survival. To determine whether Dot1L loss specifically disrupts T3 signaling, we are treating wild-type and mutant premetamorphic tadpoles with exogenous T3 and examining hallmark T3-dependent processes, including intestinal remodeling and hindlimb morphogenesis. Complementary genome-wide ChIP-seq analyses are underway to map Dot1L occupancy and delineate how its absence reshapes the chromatin and transcriptional landscape during T3-driven metamorphosis. Together, these studies will define the developmental and molecular roles of Dot1L in vertebrate metamorphosis and illuminate how histone methylation interfaces with hormone signaling to coordinate post-embryonic development.

O-046 - Thyroid hormone mediation of gonadal differentiation in zebrafish

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Sex determination and gonadal differentiation in fish are highly plastic processes regulated by genetic, environmental, endocrine, and molecular factors. Environmental variables, such as temperature and rainfall, can modify endocrine signaling pathways and influence sex differentiation in certain fish species. Thyroid hormones (THs) have been implicated in the multifactorial regulation of gonadal development in fish, with hypothyroid conditions tending to skew sex ratios toward females and promote ovarian development. However, it remains unclear whether thyroid hormones mediate environmentally induced changes during early gonadal differentiation. Changes in early gonadal differentiation in zebrafish were evaluated using three experimental groups: euthyroid controls, hyperthyroid fish treated with thyroxine (T4), and hypothyroid fish induced by methimazole, an inhibitor of thyroid hormone synthesis. Treatments were initiated at 3 days post-fertilization (dpf) and continued until adulthood (120 dpf), encompassing the critical window of gonadal differentiation (18–34 dpf). Fish were maintained either at a standard temperature (28 °C) throughout development or exposed to elevated temperature (35 °C) during the period of gonadal differentiation.Results demonstrated a significant increase in male development at 35 °C compared to 28 °C. Methimazole-treated fish exhibited delayed gonadal differentiation, indicating that disruption of thyroid hormone signaling affects developmental timing. Histological analyses further highlighted the essential role of thyroid hormones: hypothyroid conditions led to a marked reduction in spermatozoa within the testes and a decrease in the number of preovulatory (mature) follicles in the ovaries, regardless of temperature.In addition, the study examined components of the hypothalamic–pituitary–thyroid (HPT) axis, including hormone production and receptor expression. Collectively, these findings support the hypothesis that thyroid hormones play a significant role in mediating temperature- dependent sex differentiation in zebrafish.

O-047 - The hypothalamic regulation of the thyroid axis in teleosts: insights from knockout models and novel transgenic tools

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Thyroid hormones play essential roles in vertebrate physiology, regulating a wide range of processes including growth, development, metamorphosis, metabolism, and reproduction. The synthesis and release of thyroid hormones by the thyroid gland are controlled by hypothalamic factors that stimulate the secretion of thyroid-stimulating hormone (Tsh) from pituitary thyrotropes. In mammals, the release of Tsh from the pituitary gland is primarily regulated by hypothalamic thyrotropin- releasing hormone (Trh). However, in teleost fish, the hypothalamic regulation of Tsh release remains poorly understood.

To address the longstanding question of the central regulation of the thyroid axis in teleosts, we generated zebrafish *trh* and *tshβ* null mutants using CRISPR-Cas9. Unlike *tshβ* knockout fish that exhibited a severe hypothyroid phenotype, *trh*$^{-/-}$ fish did not show signs of thyroid axis dysfunction. This included non-altered *tshβ* gene expression, normal development, growth, pigmentation, and reproduction. Also, HCR RNA-FISH analysis revealed no detectable expression of the three known Trh receptors (trhra, trhrb, and trhr2) in pituitary thyrotropes, suggesting that Trh does not directly regulate thyrotropes in zebrafish. Instead, we discovered that Trh regulates hatching and stimulates Acth and Msh cells in medaka and zebrafish, indicating that the role of Trh in teleosts differs from its canonical role as a hypothalamic regulator of the thyroid axis in mammals.

To further understand the regulation of the teleost thyroid axis, we generated transgenic zebrafish and tilapia reporter lines. We first cloned a 2.9 kb 5' upstream regulatory region of the medaka *tshβ* gene into a Tol2-compatible vector. This vector was then used to generate transgenic reporter lines expressing GFP and the genetically encoded calcium indicator GCaMP6s in thyrotropes of zebrafish and tilapia. Notably, the medaka *tshβ* gene promoter is reliably activated in thyrotropes across multiple teleost species. These reporter lines enable the visualization and functional analysis of thyrotropes in real time.

These novel tools are anticipated to provide a powerful platform for investigating how thyrotropes adapt to changing physiological demands during growth and development. In addition, fluorescent labeling of thyrotropes will enable their isolation for future transcriptomic analyses, creating opportunities to identify the hypothalamic factors that regulate the teleost thyroid axis.

O-048 - Metamorphosis determines immune responses in juvenile Senegalese sole

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Thyroid hormones (THs) are essential for flatfish metamorphosis. However, their importance for immune ontogeny is unclear and the specific mechanisms linking thyroid signalling to immune ontogeny remain poorly defined. Using the Senegalese sole (*Solea senegalensis*) flatfish as a model, the influence of THs on lymphoid tissue development, immune gene regulation, and post-metamorphic immune competence was investigated. Experimental hypothyroidism was induced by exposing larvae to the thyroid peroxidase inhibitor methimazole (MMI, 0.3 mM) from 7 to 20 days post-hatch. Morphological and anatomical transitions were assessed via high-resolution light-sheet microscopy and 3D reconstruction, while transcriptome landscapes were characterized by RNA sequencing and in silico analysis of thyroid hormone response elements (TREs). The effectiveness of TH ablation with MMI was confirmed by the absence of eye migration, cranial remodelling, and skin pigmentation asymmetry. Transcriptome profiling revealed 2,509 differentially expressed genes (DEGs), that mapped primarily to metabolism, morphogenesis, and epigenetic regulation. Of the DEGs 6.25% were immune-related and were enriched in genes of innate humoral immunity, haematopoiesis, macrophage/dendritic cell function, and natural killer cell activity. Promoter analysis indicated that TH responsiveness was not strictly coupled with TRE presence and indicated that THs regulate immune targets through both direct and indirect genomic mechanisms. Juveniles that had a disrupted metamorphosis exhibited altered responses to a challenge with bacteria (formalin-inactivated Photobacterium damselae piscicida, 1×10^9 CFU. mL-1) or a viral proxy [poly(I:C), 1 mg. mL-1]. Collectively, the results establish TH signalling as a major driver of immune tissue development and immune system level maturation during flatfish metamorphosis. A modular regulatory framework through which THs shape immune development and competence, was identified and provides critical new insights into the endocrine-immune axis during vertebrate ontogeny.

Acknowledgements: This study received Portuguese national funds from FCT - Foundation for Science and Technology through institutional projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020, and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through projects EMBRC.PT ALG-01-0145-FEDER-022121 and BIODATA.PT ALG-01-0145-FEDER-022231. SCS was supported by a PhD scholarship (SFRH/BD/08486/2020) from FCT.

O-049 - DNA methylation during limb growth at *Xenopus tropicalis* metamorphosis

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Crosstalks between thyroid hormones (TH) and gluco-corticoids (GC) signaling is central for development, energy balance, and overall, for the integration of internal and external signals in sustaining homeostasis. The high sensitivity of amphibian metamorphosis to thyroid axis status, stress and environmental inputs makes it a powerfull system to address crosstalks. Remarkably, depending on the developmental time and external cues, GC can accelerate or slow down the onset of metamorphosis. What makes it that the simultaneous action of TH and GC is more (or less) than their individual action ?

In previous work, we compared the transcriptional output in various TH / GC contexts during limb growth and tail resorption of Xenopus tropicalis tadpoles, clearly pinpointing to changes in the DNA methylation dynamic machinery. We therefore profiled genome wide changes of DNA methylation with MethylCAP-Seq, and isolated and sequenced DNA with high or low methylation status.

We found a large number of Differentially Methylated Regions (DMR), highlighting the dynamic induced my hormone treatments. Almost all DMRs are located far from genes, and they strongly overlap with repeated sequences. Reflecting the DNA methylation dynamic, we also found the transcriptional signature of active transposable elements, which are usually tightly repressed. We here address the dynamic of the different compartments of methylated DNA (high and low) at repetitive sequences and promoters. Despite the limited resolution of the repetitive component of the genome, we found that low methylation DNA regions located transposable elements express long non coding RNA.

The repetitive component of the genome is difficult to probe but may hold the clues to understand the wide functional reprograming operating at metamorphosis and stress.

SOA8 - Multi-omic approaches to unravel new mechanisms of action of endocrine disruptors

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In recent decades, the effects of environmental pollution on both human and environmental health have become a major global concern. Historically, Endocrine Disruptors (EDCs) were known for affecting reproduction-related signaling mechanisms, but more recent studies have shown they also act through other hormonal systems. Among the various classes of pollutants, endocrine-disrupting chemicals (EDCs) have received particular attention due to their ability to interfere with hormonal systems. Traditionally, EDCs were primarily associated with disruption of reproductive signaling pathways; however, growing evidence indicates that their effects extend far beyond reproductive toxicity, influencing multiple endocrine axes, including metabolic, thyroid, and neuroendocrine systems. Moreover, EDCs can induce persistent biological effects through mechanisms such as DNA methylation, histone modifications, and regulation of non-coding RNAs. These modifications may have long-term consequences and even contribute to transgenerational effects, raising additional concerns for both environmental and public health. This broader spectrum of action highlights the complexity of their biological effects and underscores the need for a more comprehensive understanding of their mechanisms of action, which is crucial for protecting human and environmental health through adequate risk assessment and regulation.

Recent advancements in omics technologies (genomics, transcriptomics, metabolomics, lipidomics, etc.) have significantly improved our ability to investigate the effects of EDCs across different biological levels. These approaches enable the identification of molecular alterations that precede observable phenotypic changes, providing early indicators of toxicity. To further enhance understanding of EDCs' modes of action, research efforts should focus on several key areas. First, the identification and validation of reliable biomarkers of exposure is essential. Recent studies have shown that changes in the transcriptome, not only reveal affected signaling pathways, but also serve as early response signals to exposure to EDCs. However, consensus on transcriptomic biomarkers for (eco)toxicological characterization is still lacking. Second, the integration of multi-omics data offers is crucial for capturing the complexity of EDCs effects, providing a more comprehensive understanding of biological responses across molecular layers. Furthermore, emerging technologies, such as spatial and single-cell omics, should be further developed and implemented to address cellular heterogeneity, which is an inherent property of endocrine responses.

In summary, advancing EDC research requires the integration of innovative methodologies and multi-omics approaches to improve mechanistic understanding, strengthen risk assessment frameworks, and ultimately contribute to better protection of both human and environmental health.

Funding: Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033, Grants PID2021-122929OB-C33, CEX2018-000794-S, CNS2022-135257 and European Union (EU), Grant 101078991.

O-050 - Estrogenic activity assessment of recalcitrant pharmaceuticals found in wastewaters from Ria Formosa using the *Dicentrarchus labrax* estrogen screen (DLES) Test

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Pharmaceuticals and personal care products are persistent environmental contaminants that enter aquatic systems through wastewater treatment plant effluents. Among these, compounds with endocrine-disrupting activity pose particular concern for aquatic organisms, yet the tools used to assess estrogenic risk are largely calibrated to human estrogen receptors and overlook the distinct receptor repertoire and biology of marine vertebrates such as fish, which in addition face lifelong exposure and possible bioaccumulation and biomagnification of environmental pollutants. The estrogenic activity of wastewater treatment-recalcitrant pharmaceuticals on fish-specific estrogen receptors remains largely uncharacterised.

Here we show that several treatment-recalcitrant pharmaceuticals detected in wastewaters at the Ria Formosa coastal lagoon (Portugal), can activate nuclear estrogen receptors of European sea bass *(Dicentrarchus labrax)*. A 3-year seasonal monitoring campaign across the Ria Formosa wastewater treatment plants and environmental waters identified five highly recalcitrant compounds (carbamazepine, metoprolol, bezafibrate, azithromycin, and sulfapyridine) that persist through wastewater treatment and were detected at low to trace levels in the environmental waters. Using the DLES reporter gene assay, we evaluated the activation of the three nuclear sea bass estrogen receptors (sbEsr1, sbEsr2a, and sbEsr2b) by a concentration range (10^{-11} to 10^{-7} M) of each recalcitrant compound. Azithromycin, metoprolol, bezafibrate, and sulfapyridine significantly activated at least one of the receptors above the detection threshold, while carbamazepine showed no significant activation of sea bass nuclear estrogen receptors. The most sensitive receptor, sbEsr2b, was activated by all four compounds, with metoprolol and sulfapyridine eliciting transactivation responses across multiple concentrations. Bezafibrate additionally activated sbEsr1 and sbEsr2a at 10^{-7} M and was the only compound to recruit these two receptors. These findings support the integration of marine species-specific bioassays as complementary screening tools in environmental hazard and risk assessment frameworks for pharmaceutical pollutants in coastal ecosystems.

Acknowledgements: This study received Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025, LA/P/0101/2020; Portugal-France bilateral cooperation (PESSOA) project 2024.07223.CBM; researcher contract DL57/2016/CP1361/CT0015 to PP and PhD grant 2023.05271.BDANA to FC (https://doi.org/10.54499/2023.05271.BDANA). Monitoring surveys campaigns were supported by Coca-Cola Mares Circulares 2023 MicroWaste and FAM foundation MicroFish grants awarded to PP and by Águas do Algarve/CCMAR staff.

O-051 - Nuclear estrogen receptors of European sea bass (*Dicentrarchus labrax)* as targets of endocrine disrupting compounds: effects on reproduction and risk assessment potential

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Introduction: Estrogens regulate key processes in teleost reproduction through nuclear estrogen receptors (Esr), which act as transcription factors controlling gene expression. In teleosts, three Esr subtypes have been identified: Esr1, Esr2a, and Esr2b, the latter two arising from a teleost-specific genome duplication. These receptors exhibit distinct binding affinities and expression patterns, suggesting subtype-specific roles during gametogenesis along the brain-pituitary-gonad axis. Endocrine-disrupting compounds (EDCs) can interfere with endocrine regulation by acting as agonists or antagonists of these receptors. Their presence in aquatic environments and aquaculture feeds raises concerns regarding reproductive function. This study aimed to characterize nuclear Esr subtypes in European sea bass, evaluate their responses to EDCs, and assess their potential as *in vitro* bioassay tools for risk assessment.

Material and methods: Expression of esr1, esr2a, and esr2b was analyzed throughout the reproductive cycle in gonad and pituitary using qPCR. Receptor localization was assessed by immunohistochemistry. Functional characterization was performed using transient transactivation assays in HEK293 cells with an ERE-luciferase reporter system. The effects of two EDCs, genistein and fluoxetine, were evaluated across Esr subtypes. Based on these results, stable HEK293 clones expressing each receptor were generated via antibiotic selection. Clones were validated using 17β-estradiol (E2), and dose-response assays were conducted to determine EC50 and Emax values. Finally, feed samples were tested to evaluate the bioassay potential of these cell lines.

Results and Discussion: All three Esr subtypes were highly expressed in reproductive tissues, showing subtype- and stage-specific patterns across the reproductive cycle. Immunohistochemical analysis confirmed differential localization along the pituitary-gonad axis. Functional assays revealed distinct ligand affinities among Esr subtypes, resulting in differential responses to genistein and fluoxetine.

Stable HEK293 clones expressing each Esr subtype were successfully developed and validated, showing dose-dependent responses to E2 comparable to transient assays. These clones demonstrated sensitivity and reproducibility, allowing detection of estrogenic activity in feed samples. The observed responses confirmed their reliability as bioassay tools for assessing EDC activity.

Conclusion: The three nuclear estrogen receptors in European sea bass exhibit non-redundant roles in reproductive regulation. Their differential responses to EDCs highlight their relevance as molecular targets of endocrine disruption. The development of stable Esr-expressing cell lines provides a robust *in vitro* platform for detecting and evaluating estrogenic compounds, with important applications in environmental monitoring and aquaculture risk assessment.

Funding: Funded by MCIN/AEI/10.13039/501100011033/ and by ERDF a way of making Europe (PID2021-122929OB-C32) and by Generalitat Valenciana (CIAICO/2022/002). J.R. was supported by a research contract from “Programa Investigo” Generalitat Valenciana (INVEST/2023/560).

O-052 - Bridging scales in neurotoxicity: multi-omics evaluation of cadmium and ciprofloxacin effects in European seabass (*Dicentrarchus labrax*)

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Aquatic ecosystems are under increasing pressure from contaminants of emerging concern, including metals and pharmaceuticals, which are often simultaneously present in coastal environments. Among these, cadmium (Cd) and the fluoroquinolone antibiotic ciprofloxacin (CIP) are frequently detected, yet their potential neurotoxic effects in marine fish remain poorly understood, particularly across levels of biological organisation. Adopting a systems toxicology perspective, this study combines biochemical, behavioural, and multi-omics approaches to investigate neurotoxicity in juvenile European sea bass (*Dicentrarchus labrax*) following a 21-day dietary exposure.

Fish were exposed under controlled conditions to environmentally relevant concentrations of Cd (10 and 100 $mg \cdot kg^{-1}$ feed) and CIP (1 and 10 $mg \cdot kg^{-1}$ feed). Neurotoxicity was evaluated through a suite of endpoints including antioxidant defenses (SOD, CAT, GST, GPx, GSHt) and oxidative damage (lipid peroxidation) in liver and brain, acetylcholinesterase activity (AChE) in brain and muscle, and neurotransmitter levels, DNA methylation and transcriptomic responses in brain to enable multi-layered interpretation of stress responses. To assess behavioural outcomes, a battery of behavioural tests and endpoints were also evaluated.

Our results showed that Cd caused strong, multi-level hepatotoxicity characterised by oxidative stress, enzyme dysregulation, metabolic impairment, and inflammatory damage, whereas CIP showed limited tissue accumulation and induced milder, primarily metabolic effects. In the brain, both contaminants reduced glutathione levels, suggesting increased sensitivity to oxidative stress. Cd exposure led to enhanced SOD activity and a slight increase in lipid peroxidation at the higher dose, whereas CIP induced comparatively minor changes in these oxidative stress markers. AChE activity increased in the brain across treatments, indicating alterations in cholinergic signaling, while remaining unchanged in muscle. Behavioural assessments revealed reduced locomotor activity, increased freezing events, and enhanced shoal cohesion, consistent with anxiety-like responses and altered neural function.

Integration of methylomic and transcriptomic data is ongoing and aims to connect molecular level changes with physiological and behavioural outcomes, contributing to the identification of adverse outcome pathways. Overall, this multi- level approach provides a comprehensive evaluation of neurotoxicity and underscores the value of combining omics with organismal endpoints to improve mechanistic understanding. Linking responses across biological levels enhances the ecological relevance of these findings and supports more informed ecological risk assessment by helping to predict how contaminant exposure may translate into functional impairments at the organism and potentially population level in marine environments. Acknowledgments: EU project EPIBOOST - DOI: 10.3030/101078991; SP projects PID2021-122929OB-C31 and PID2021-122929OB-C33 financed by MCIN/ AEI/10.13039/501100011033.

O-053 - Chemically-induced molting disruption in arthropods – an issue of increasing environmental concern

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Molting is a central developmental process in arthropods, regulated by coordinated endocrine, neuroendocrine, metabolic, behavioral, and structural pathways. These pathways are susceptible to disruption by chemicals that interfere with hormonal signaling, cuticle turnover, chitin synthesis, neuropeptide activity, or gene regulatory networks. Because molting disruption can arise through diverse and often poorly resolved mechanisms, new mechanistically anchored tools are needed to identify key events underlying toxicity and to detect molting disruptors across a broad range of arthropod species and chemicals.

This project, co-financed by the Partnership for the Assessment of Risk from Chemicals (PARC), aims to establish an integrated scientific framework to characterise molting disruption using model organisms and wildlife species. The work involves sequential steps starting with a systematic review, refinement, and expansion of existing molting-relevant Adverse Outcome Pathways (AOPs). Conserved molecular initiating events and key events in the AOP as well as evolutionary conserved pathway linkages will be mapped and evaluated across taxa, while also identifying knowledge gaps for targeted experimental investigation.

Building on this mechanistic foundation, a suite of bioassays has been developed and will be harmonised across laboratories to quantify molecular, cellular, phenotypic, behavioral, and organism-level responses to stressors. Approaches include transcriptomics, targeted gene expression analyses, metabolomics, hormone quantification, behavioral profiling, and advanced *in vitro* and *in vivo* New Approach Methodologies (NAMs). Computational x-species comparisons will support identification of conserved responses and improve extrapolation across species groups.

The resulting toolbox will be applied to environmentally relevant chemicals to identify compounds capable of disrupting molting physiology and related biological pathways. Multi-endpoint and multi-species datasets will be integrated to construct quantitative AOPs (qAOPs), enabling the development of predictive models and quantitative key event relationships describing how early molecular perturbations propagate to organism-level adversity for use in Next Generation Risk Assessment.

This presentation will summarise the scientific foundation, introduce the methodological approaches, and present results from initial steps of the work (i.e. AOP-definition, bioassay/NAMs development, reference chemical testing).

Acknowledgement: This work was supported by the Research Council of Norway project EXPECT (RCN-315969), EU-HEU project PARC (#101057014), and NIVA's Computational Toxicology Program, NCTP (RCN-342628).

Keywords: Molting disruption; Arthropods; Adverse Outcome Pathways (AOPs); New Approach Methods (NAMs); Quantitative AOPs (qAOPs)

O-054 - Cellular landscape of tributyltin toxicity using single-cell and spatial transcriptomics in zebrafish embryos

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Tributyltin (TBT) is a highly toxic and persistent endocrine-disrupting compound with well-documented adverse effects on aquatic organisms, particularly impacting the development and function of the nervous system and visual structures. However, the cellular and tissue-specific mechanisms underlying these effects remain poorly understood. This limitation is particularly relevant in small vertebrate models such as zebrafish embryos, where bulk approaches can obscure localised responses. Here, we investigated TBT effects in zebrafish embryos *(Danio rerio)* using single-cell and spatial transcriptomics, capturing cellular heterogeneity following 24 h exposure to environmentally relevant concentrations (3 nM and 30 nM).

Single-cell analysis was performed using NextGEM GEM 3' v3.1 (10X Genomics), which yielded a total of 13700 high-quality cells. Unsupervised clustering identified 28 clusters, of which 15 exhibited differential gene expression between control and TBT-exposed cells. Neuronal and neural progenitor populations were highly impacted (1025 and 436 differentially expressed genes (DEGs), respectively), and most affected pathways were associated with the electron transport chain and cell death. Additionally, eye-related clusters showed a marked reduction in photoreceptor (rods and cones) and retinal cells at 30 nM, indicating impaired cell survival. Retinal cells showed 75 DEGs associated with circadian rhythm and metabolic processes.

MERFISH-based spatial transcriptomics (MERSCOPE®, 1000-gene panel) further supported these findings. A total of 275795 cells were obtained, distributed across 35 clusters, enabling high-resolution identification and spatial localisation of neural populations. Differential expression analysis revealed cell-type-specific responses to TBT exposure, with broad upregulation in glutamatergic neurons and pronounced downregulation in monoaminergic populations at the highest concentration.

Altogether, this work highlights the neurotoxic and retinotoxic potential of TBT at environmentally relevant concentrations and underscores the value of integrating single-cell and spatial transcriptomic approaches to uncover cell-type-specific mechanisms of endocrine disruption that may remain undetected by conventional toxicological approaches.

Funding: Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033, Grants: PID2021-122929OB-C33, CEX2018-000794-S

O-055 - Chronic exposure to environmentally relevant PFAS mixtures disrupts metabolic activity and tissue organization in human hepatic organoids

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The liver is a central endocrine and metabolic organ that integrates hormonal and nutritional cues to maintain whole-body energy homeostasis. Owing to its dual role as a key regulator of metabolic and endocrine processes and the principal site of xenobiotic biotransformation, it is a primary target of environmental contaminants, which can interfere with hepatic signaling pathways and metabolic regulation. Among contaminants, the per- and poly-fluoroalkyl substances (PFAS) are persistent environmental and occupational chemicals with potential endocrine-disrupting activity. Human exposure generally occurs through complex mixtures and their effects on human hepatic tissue are still not fully understood, especially under chronic low-level exposure conditions representative of environmental and occupational settings. In this study, primary human hepatic organoids were used as a 3D model to investigate the effects of a PFAS mixture composed of perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS) and hexafluoropropylene oxide dimer acid (HFPODA or GenX). The mixture composition and nominal concentrations were defined based on biomonitoring data reported in the literature for high-exposure scenarios, including occupational settings. In addition, a preliminary screening employing HepG2 cells, integrated with benchmark concentration analysis, was conducted to guide the selection of toxic concentrations compatible with prolonged exposure to be applied to organoids. Human hepatic organoids were exposed to the selected mixture during proliferation (first 7 days of culture) followed by differentiation phases (additional 10 days of culture). The experimental design integrates Cell Titer-Glo® 3D assay, morphometric evaluations and histological staining, with the aim of analyzing metabolic activity, organoids growth and organization, cellular monolayer thickness, lipid accumulation and extracellular matrix deposition. A pilot phase conducted with a low-concentration mixture showed morphometric and histological alterations in organoids, including changes in cellular monolayer thickness, increased lipid accumulation and extracellular matrix remodeling. Based on these findings and benchmark concentration analysis, a higher-concentration mixture was subsequently evaluated. Preliminary results indicate a reduction in metabolic activity, as determined by the Cell Titer-Glo® 3D assay. Further functional, morphometric, and histological analyses are currently underway. Overall, this study supports human hepatic organoids as an advanced experimental model to investigate early effects of contaminants of emerging concern with endocrine activity such as PFAS mixtures on human liver tissue. The use of a 3D model allows the integration of functional, endocrine, morphometric and histological endpoints, providing an approach closer to hepatic physiology than conventional cell models. This study contributes to elucidating the effects of prolonged PFAS exposure on metabolic function and structural integrity of human hepatic organoids, providing insights relevant to human, environmental and occupational exposure scenarios.

O-056 - Endocrine and molecular disruption in sea urchin larvae (*Paracentrotus lividus*) exposed to citalopram and triclosan under climate change stressors

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The widespread detection of pharmaceuticals and personal care products (PPCPs) in coastal environments has raised significant concern regarding their capacity to induce non-classical endocrine disruption in marine invertebrates. Among these, the selective serotonin reuptake inhibitor citalopram (CIT) and the antimicrobial agent triclosan (TC) are frequently quantified at environmentally relevant concentrations, where they can interfere with neuroendocrine, metabolic, and signalling pathways crucial for development. Concurrently, global climate change drives ocean warming (OW) and ocean acidification (OA), which may interact non-linearly with pollutants, potentially exacerbating their toxicity. Using the early life stages of the sea urchin *Paracentrotus lividus* as a sentinel model, this study evaluates how CIT and TC interact with predicted climate stressors at both phenotypic and transcriptomic levels, focusing on the disruption of molecular cascades governing homeostasis. Fertilized eggs of *P. lividus* were incubated for 48 hours post-fertilization under control conditions and exposure to the EC10 values of CIT (46.70 µg/mL) and TC (98.92 µg /mL), individually and combined with climate scenarios. Projected 50-year climate scenarios were simulated, inducing OA by increasing dissolved inorganic carbon (+126 µmol/kg DIC) and OW by a temperature elevation from 20°C (control) to 24°C. Water-chemistry parameters were calculated using the seacarb package in R. At the pluteus stage, morphological growth was assessed via light microscopy. Concurrently, RNA-seq was performed (log2FC ≥ |2|, Padj ≥ 0.05 via DESeq2) followed by Gene Ontology functional enrichment analysis to identify affected molecular networks. Phenotypically, isolated OA and OW treatments did not alter larval growth compared to the control. Single exposure to CIT and TC resulted in a significant biometric reduction of approximately 10–20%. However, when combined with OA, a distinct synergistic toxicity emerged, reducing larval growth by 20% under CIT and up to 50% under TC, demonstrating that acidification acts as a potent stress-potentiator. Conversely, OW temporarily offset these biometric deficits, bringing growth back to control levels through an intensive transcriptional shift toward ribosomal biogenesis and protein translation. The transcriptomic profiles provided deep mechanistic evidence of endocrine and neurodevelopmental disruption, even when morphological endpoints remained unaffected. CIT alone acted as a neuroendocrine disruptor, dysregulating genes associated with neurodevelopment, macromolecule metabolism, and apoptosis. When combined with OA, it caused a severe collapse in morphogenetic signalling, skeletal pattern formation, and negative regulation of growth. Under OW, CIT shifted cellular resources toward calcium-mediated signalling dysregulation and transcriptional control. On the other hand, TC exposure heavily targeted metabolic and structural homeostasis, dysregulating genes involved in ATP synthesis, calcium signalling cascades, cytoskeletal organization, and ciliary motility. When combined with OW, TC triggered a broader transcriptional response affecting embryo development, extracellular matrix organization, and apoptosis. Under the multi-stressor scenario (Contaminant + OW + OA), both chemical pathways converged into a shared systemic alarm response. This complex profile was characterized by a severe dysregulation of intracellular signal transduction, calcium signalling dysregulation, protein phosphorylation, and immune defence mechanisms. In conclusion, our findings underscore these low concentrations of PPCPs act through non-classical endocrine networks to compromise early

echinoderm development. While warming can temporarily mask biometric impacts, the underlying transcriptomic data reveals a profound physiological shift from a regular "growth mode" to a critical, energy- demanding "survival mode". Integrating these multi-stressor mechanistic biomarkers into predictive ecotoxicological risk assessments is essential to safeguard marine population recruitment under accelerating global change.

O-057 - Non-EATS modalities in human and environmental endocrine disruption research

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The identification of Endocrine Disrupting Chemicals (EDCs) has historically focused on the EATS (Estrogen, Androgen, Thyroid, and Steroidogenesis) modalities. However, growing evidence links chemical exposure to non-EATS pathways, including those leading to metabolic diseases such as obesity and related metabolic disorders. Current EU regulations assess ED endpoints as hazard classes, such as via the Classification, Labelling and Packaging regulation (Categories 1 and 2 for both human health and the environment). Such hazard assessments rely primarily on validated and regulatory-accepted testing guidelines which are currently not in place for non-EATS modalities; yet, the European Chemicals Agency (ECHA) has specifically identified metabolic disorders as key regulatory challenges. Our group is working on the development of new approach methodologies (NAMs) for these endpoints as well as research to better understand which mechanism are involved. At our lab we focus in particular on obesity and fatty liver disease. For obesogenic effects, the interaction between adipogenesis and Peroxisome Proliferator-Activated Receptor gamma (PPAR gamma) activation, a master regulator of lipid metabolism is of interest. Ongoing work on validation of adipogenesis as well as PPAR gamma transactivation assays will be presented. Using these assays, work on legacy and emerging contaminants, including bisphenol alternatives and per- and polyfluoroalkyl substances will be shown. For fatty liver disease, we use zebrafish as a model to study Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). Zebrafish offer high physiological conservation of lipid processing and allow for real-time visualization of hepatic lipid accumulation, providing a powerful whole-organism perspective on how EDCs accelerate the transition from simple steatosis to advanced liver pathology. The transition to non-animal approaches for non-EATS modalities is not merely a replacement strategy but a requirement for next generation risk assessment. However, these NAMs must be developed within a One Health framework in mind. Some mechanisms might be conserved, yet some species differ in their metabolic rate, receptor binding affinities, and toxicokinetic profiles, and a "one-size-fits-all" model might not always work. By integrating comparative toxicological data across species, we can develop predictive tools that protect both human and ecosystems. Only by aligning our molecular understanding of metabolic disease and its conservation between species, can we effectively mitigate the rising tide of chemically induced metabolic disorders.

SOA9 - Induced primordial germ cells (iPGCs) and expanded germline stem cells (eGSCs) for high-efficient fish surrogate reproduction

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Surrogate reproduction via the transplantation of primordial germ cells (PGCs) or germline stem cells (GSCs) holds significant promise for advancing developmental biology and genetic breeding. However, its efficiency remains low due to the limited availability of donor PGCs and GSCs. First, we develop a novel approach that uses 9 germplasm factors (9GMs) to reprogram zebrafish blastoderm cells into induced PGCs (iPGCs), enabling the production of functional gametes through iPGC transplantation. Remarkably, zebrafish-derived 9GMs were able to convert blastula cells of other fish species into iPGCs, facilitating the generation of xeno-gametes in iPGC-transplanted host fish. Second, we demonstrate that a mutation in the Leydig cell-specific cyp11a2 promotes unlimited proliferation of GSCs, enabling the expansion of xeno-GSCs in *cyp11a2*

mutant zebrafish. These expanded GSCs (eGSCs) can be efficiently transplanted into GSC-deficient *nanos2* $^{-/-}$ host fish, achieving highly efficient surrogate reproduction. Collectively, our study develops novel and efficient methods for generating iPGCs and eGSCs in teleosts, offering great potential for basic research and aquaculture applications.

O-058 - CRISPR disruption of mineralocorticoid receptor does not affect overall tadpole growth and development and shows aldosterone unnecessary for metamorphosis

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Activation of the glucocorticoid receptor (GR) plays a vital role in potentiating thyroid hormone (TH) action on target organs during frog metamorphosis. However, the contribution of mineralocorticoid receptor (MR) in metamorphosis remains unclear. During metamorphosis expression levels of MR increase as well as plasma levels of the MR ligands corticosterone (CORT) and aldosterone (ALDO), and thus we hypothesized that MR contributes to TH-driven metamorphic progression. To examine the role of MR in metamorphosis, we used CRISPR/Cas9 to generate MR loss-of-function mutants in Xenopus tropicalis. MR- deficient tadpoles exhibited normal growth or development from larval stages through metamorphic completion and survived to become fertile adults. In addition, GR/MR double-knockout tadpoles did not exhibit exacerbated growth or development defects compared to GR single-knockout animals. To further assess MR function, we performed RNA-seq on cultured GR mutant tadpole tails treated with ALDO and/or TH. Even though differential expression analysis identified hundreds of TH-response genes, no gene was found to respond to ALDO. Collectively, our findings indicate that MR is not required to regulate overall growth, development, or tail gene expression in tadpoles but does not rule out non-vital tissue- specific actions. In addition, because ALDO does not bind GR under physiological conditions, CORT is the only corticosteroid required for tadpole metamorphosis.

O-059 - Regulation of zebrafish early sex differentiation

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Zebrafish has been widely used in research, but the mechanisms controlling its sex determination and gonadal development are still unclear. We examined zebrafish gonadal gene expression and sex differentiation at different stages of development, from 8, 12 14, 21, and 26 dpf (days post fertilization), and found that the earliest sign of sexual dimorphism is the increased IGF1 and pAkt in the presumptive female gonad. This is followed by the presence of bigger female gonads at 12 dpf. These results imply that the PI3K/Akt pathway is probably involved in female gonadal differentiation. Hormonal control may come later, with large amounts of cyp19a1a expression and estrogen production for future females at 3 weeks of age. Furthermore, our single cell RNAseq analysis identified genes expressed during zebrafish ovarian development and genes that may be involved in male development. It also reveals genes that participate in the differentiation of females versus males during sex differentiation.

O-060 - Complementary and additive functions of TRα and TRβ in regulating larval cell death and adult stem cell development during Xenopus intestinal metamorphosis

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Intestinal structure is drastically changed from the fetal to adult form during postembryonic development, a period around birth in mammals. This process is regulated by thyroid hormone (T3) via its receptors, T3 receptor (TR) α and TRβ. We have been studying intestinal remodeling during Xenopus tropicalis metamorphosis as a model for human postembryonic adult organ maturation. We have used chromatin immunoprecipitation (ChIP)-seq analyses of the intestine from wild type, TRα- or TRβ-knockout premetamorphic tadpoles treated with or without T3 and identified many TR-bound genes. The results reveal that individual TR knockout reduces both the number of TR-bound genes and the extent of TR binding to target genes with TRα knockout has much more dramatic effects than TRβ knockout. On the other hand, the TR-bound genes are largely common among the three genotypes. In addition, by using gene editing, we have knocked out TRa and TRb individually or together. We have observed that knocking out of either TR genes causes various developmental defects, including inhibiting intestinal remodeling but the tadpoles can nonetheless complete metamorphosis. On the other hand, knocking out both TR genes leads to tadpole lethality at the metamorphic climax and prevents larval epithelial cell death and formation of adult stem cells during intestinal metamorphosis. These findings suggest that both TRα and TRβ contribute to the binding of target genes mostly common to both TRs, with TRα having a bigger contribution, in the intestine and that they complement each other to ensure proper temporal completion of adult intestinal development.

O-061 - Elucidating the function of the thyroid hormone receptor alpha a (thraa) in zebrafish heart regeneration

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Zebrafish are an excellent genetic model for studying both vertebrate development and tissue regeneration, and can fully regenerate the heart after various types of injury. Thyroid hormone (TH) signaling was reported to promote cardiomyocyte (CM) maturation in endothermic animals. Elevated TH levels, coupled with increased basal metabolism, promote CM cell cycle exit and polyploidization, thus limiting heart regenerative potential. We investigated the molecular mechanisms of TH signaling in heart regeneration using a time-course sequencing experiment. We assessed heart regeneration capacity in thyroid hormone receptor alpha a (thraa) mutant zebrafish, which carry an 8-bp insertion that leads to truncation of the Thraa protein and impairs TH signaling. The thraa +8 bp mutant zebrafish exhibited an enhanced heart regenerative response. Our study showed that, in *thraa*+/– mutants, a transiently augmented inflammatory response and an extended CM proliferative window are associated with metabolic switches across different phases. Moreover, we found that thraa transcriptionally regulates *hypoxia-inducible factor 3 subunit alpha* (*hif3a*), and its knockout in zebrafish impairs heart regeneration. In conclusion, our findings demonstrate that TH signaling via Thraa modulates zebrafish heart regeneration through coordinated regulation of metabolism, inflammation, and cardiomyocyte proliferation.

O-062 - Thyroid hormone receptor regulates cellular senescence to affect larval tissue degeneration in *Xenopus tropicalis*

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Thyroid hormone (T3) is a critical regulator of development, health, and aging. Increasing evidence suggests that T3 regulates cellular senescence through T3 receptors (TRs). However, it is difficult to the role and regulation of cellular senescence during mammalian postembryonic development when plasma T3 level peaks, in part due to maternal influence. Anuran metamorphosis, which mimics mammalian postembryonic development and is also regulated by T3 but independent of maternal influence, is an excellent model to study T3-dependent developmental cellular senescence. Here we use TR double knockout (TRDKO) Xenopus tropicalis tadpoles lacking both TRa and TRb, the only two receptor genes in vertebrates, to investigate if and how T3 regulates cellular senescence during metamorphosis. By using RNA-seq analyses, we found that cellular senescence pathway was highly enriched among T3-upreguleted genes in tail of wild type but not TRDKO animals. Of interest, the cell cycle inhibitor p15 was found to be highly induced by T3 in tail during both natural and T3-induced metamorphosis. In addition, cdk1, a cyclin-dependent kinase, known to be involved in cellular senescence and also important for larval tissue cell death, was strongly upregulated by T3 in tail during metamorphosis. Our findings for the first time reveal that T3 induces cellular senescence in the tail prior to significant tail resorption during metamorphosis, most likely via regulating the expression of both cell cycle activators and inhibitors to induce cell cycle arrest and further suggest that cellular senescence may be an early step or prerequisite for cell death during larval tissue degeneration.

O-063 - A somatostatin of maternal origin is crucial for early development

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Somatostatin (SST) was first discovered in sheep hypothalami as a 14-amino acid peptide antagonist of growth hormone- releasing hormone. Since then, new isoforms have been found and shown to be produced in other organs, including d-cells in the pancreas and gastric tract. SST has been shown to have multiple roles, including inhibition of cell proliferation and metabolic regulation, which are largely conserved among vertebrates. However, while mammals have two SST family genes, teleost fishes have six, including several isoforms, and their roles have only been partially explored. We have produced CRISPR/Cas9 genome-edited mutant lines for zebrafish SST genes and characterised their phenotypes to uncover their functions.

We found zebrafish *sst6.2* is a maternally transmitted RNA essential for early development. *Sst6.2* affects calcium waves, F- actin polymerisation and cell division. Most *sst6.2*-deficient zebrafish embryos fail to develop; most die within 24 hours and can be rescued by *sst6.2* mRNA. *Sst6.2*-deficient embryos have delayed maternal RNA degradation and fail to activate the zygotic genome, largely because of reduced phosphorylation of SMAD1/5 and STAT3. Since *sst6.2* is maintained in fish, amphibians, and reptiles and appears to have been lost in mammals, we suggest it is an essential developmental RNA for animals with lecithotrophic and matrotrophic development.

Acknowledgement: This work was funded by the Shanghai Municipal Government to Shanghai Ocean University and by the FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

O-064 - Spontaneous maturation of female American eels (*Anguilla rostrata*) in freshwater ponds

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Eels of the genus Anguilla are catadromous fish species characterized by spawning migration from continental freshwater and brackish habitats to oceanic spawning grounds, where mature eels have historically been found only in seawater. In this study, we reported the spontaneous maturation of female American eels *(Anguilla rostrata)* in freshwater aquaculture ponds. Nowadays, the production of *A. rostrata* in China accounts for half of the total eel production. In May 2025, 6 females with swollen belly and black pectoral fins were collected by screening over 150,000 eels in freshwater ponds with water temperature at 25°C. Statistical analysis of five of these eels revealed that the average body length of these six eels was 56.3±2.9 cm, and the average body weight of these was 455.6±74.5 g. We selected 5 fish for sampling analysis, and 1 fish for artificial propagation. The GSI of these five eels ranged from 10.9% to 50.1%. The oocyte diameter of female with the highest GSI is 753.2±27.9 um. The analysis of the otoliths revealed the 5 females were 2 years old, and the strontium element was only present in the center of the otoliths. Two female contained contents in their digestive tract. The concentration of A-Linolenic acid (ALA) ($P < 0.05$) and Arachidonic acid (ARA) showed negative and positive correlation with GSI, respectively. The analysis of brain transcriptomics indicated that the amhr-II mRNA exhibited significant increase during the early vitellogenic stage. RT-qPCR analysis revealed that the cyp19a1 mRNA in ovaries and the lhb mRNA in pituitary gland significantly increased only during the migratory nucleus stage. Pituitary fshb mRNA begins to be expressed when oocytes reach the oil-droplet stage, peaks during the early vitellogenic stage, and then declines as oocyte development proceeds further. Meanwhile, the vtg gene in the liver begins to be expressed during the mid vitellogenic stage and reaches its peak during the migratory nucleus stage. Furthermore, through routine hormone injections to artificially stimulate maturation in one of the maturing females without sampling, it was found that only two injections were required for its Body Weight Index to exceed 105%, reaching a state ready for spawning induction. Finally, hybrid larvae were successfully obtained by mating the mature female American eel with mature male Japanese eels *(Anguilla japonica)*. These preliminary observations provide essential natural reference data for research on the reproductive maturation of eels.

SOA10 - Endocrine responses to environmental challenges: an overview

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Endocrine signalling regulates most of the biological processes in animals and there is a close link between external (environmental) signals and the hormonal responses in animals to better align survival of the animal within the changing environment. In the past, such environmental changes were relatively predictable over time, e.g. diurnal changes and seasonal changes, and animals were well-adapted to respond accordingly physiologically, or to avoid the environmental challenge by avoidance (i.e. migration, hibernation, diapause, torpor). In the modern world with serious anthropogenic impacts, including pollution (chemical, noise and light), habitat competition and climate change (global warming, precipitation changes, temperature increase, disruption of seasonal patterns), the natural rhythms and responses are destroyed, and survival of both vertebrate and invertebrate life is in serious peril.

The theme of this symposium is on endocrine responses to environmental challenges and this lecture will provide an overview on the topic on which shorter lectures will build.

In vertebrates, the hormonal response to environmental challenges primarily involves the hypothalamus-pituitary-adrenal (HPA) axis with the hormone cortisol, and the sympathetic nervous system (with adrenaline), which alters metabolism, growth, and immune function to ensure survival. These systems adapt to stressors like climate change, toxins, and pollutants, which can cause chronic dysfunction, such as metabolic disorders or reproductive issues.

Insects, as the largest of group of invertebrates on earth, have developed sophisticated endocrine systems to manage environmental challenges, enabling them to survive in diverse habitats ranging from arctic permafrost to hot deserts. The primary endocrine response involves modulating metabolic activity, developmental timing, and behaviour through hormones and neurohormones in response to abiotic (temperature, light, water) and biotic (nutrition, crowding) factors. Key regulators include juvenile hormone (JH), ecdysteroids (e.g., 20E), insulin-like peptides (ILPs), and adipokinetic hormone (AKH). Close timing of biological processes like finding a mate, egg-laying, embryonic development, access to nutritional foods and to seasonal changes are now perturbed due to man-made disturbances in the climate system and habitats. In such cases, the biological clock is no longer synchronised with the environment and despite hormonal signalling, the animal group can no longer pivot, and numbers are in serious decline. In this lecture, examples and mechanisms of hormonal response to environmental stressors such as temperature extremes, metabolic and nutritional stressors, water loss, diapause, immunity and reproduction trade-offs will be presented.

O-065 - Brown trout hepatocyte spheroids: an alternative 3D platform for assessing endocrine and climate-related stressors

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The adoption of New Approach Methodologies (NAMs) has been promoted by European regulatory agencies to support ethical experimental aligned with the 4R principles, minimizing the use of animals in research. In this context, *in vitro* cell- based assays have been implemented, but conventional two-dimensional (2D) cell culture show limited *in vivo* physiological relevance and predictive capacity.

Therefore, three-dimensional (3D) *in vitro* models, such as spheroids, advance in the reliability and translational value of experimental models. These models more accurately mimic tissue organization, cell–cell interactions, and functional signalling pathways observed *in vivo*. While 3D models are well established in mammalian systems, the implementation and validation of these approaches in fish models have only recently gained attention.

Our group has been focusing on the establishment and validation of a brown trout primary hepatocyte spheroid model for ecotoxicological applications as an innovative and ethically sustainable experimental platform. Brown trout hepatocyte spheroids were morphologically and functionally stable from approximately day 12 in culture and remained viable for nearly one month. The present study used the model to assess the effects of the endocrine disruptor 17α-ethinylestradiol (EE2), and a +3 °C warming scenario. Spheroids were cultured at 18 °C and 21 °C for 25 days and subsequently evaluated for metabolic activity, biometric parameters, and ultrastructural features.

EE2 at environmentally relevant concentrations altered classical estrogen-responsive pathways, without compromising spheroid viability or morphometric parameters. Regarding temperature effects, a +3 °C warming scenario accelerated cell aggregation kinetics from the earliest stages of culture. This resulted in larger spheroid diameter and area at 21 °C than at 18 °C, although no changes in cytotoxicity were observed. Ultrastructurally, spheroids maintained at 18 °C and 21 °C were comparable. In both conditions, a progressive increase in dense bodies suggestive of autophagic activity was observed over time in culture.

Overall, our brown trout hepatocyte spheroid model showed robustness and physiological relevance for assessing endocrine- disrupting and climate-related thermal stressors. The maintenance of viability and ultrastructural integrity supports the suitability of this 3D platform for long-term ecotoxicological studies. These findings support the growing application of fish- derived 3D *in vitro* models as reliable and more ethical alternatives for environmental risk assessment.

Funding: ATLANTIDA II (NORTE2030-FEDER-01799200), co-financed by the European Union through the NORTE 2030 Regional Program and the European Regional Development Fund (ERDF); projects UID/04423/2025 (DOI: 10.54499/UID/04423/2025), UID/PRR/04423/2025 (DOI: 10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (DOI: 10.54499/LA/P/0101/2020) financed by FCT—Fundação para a Ciência e a Tecnologia, I.P., and the European Commission's Recovery and Resilience Facility.

O-066 - Embryonic thermal programming modifies thyroid-driven metamorphosis in gilthead sea bream and European sea bass

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Thermal conditions during embryonic development can induce lasting endocrine changes that influence later development. In teleosts, thyroid hormone-driven metamorphosis remodels key tissues and represents a critical window where early environmental cues can produce long-term phenotypic effects. We investigated whether early thermal exposure (fertilisation to mouth opening) affects metamorphic remodelling and thyroid-related responses in *Sparus aurata* and *Dicentrarchus labrax*. Eggs were incubated at species-specific temperatures (sea bream: 17, 20, 23 °C; sea bass: 14, 17, 20 °C) before being transferred to a common temperature and reared under identical conditions. Sampling was performed at the end of larval rearing and mid-metamorphosis. We analysed the histology of the thyroid gland, gills, intestine, and skin, and whole- body growth. Transcripts linked to thyroid signalling, growth, and tissue remodelling were analysed. The strongest thyroid morphological response occurred at 17 °C in both species, although the downstream developmental effects were species- specific. In sea bream imprinted at 17 °C compared to other temperatures, the thyroid follicle number and follicle perimeter were highest, gill filaments were longer, gill mucous cell density was higher, and intestinal perimeter, villi length and skin goblet cells were larger. In sea bream, the change in phenotype (17 ºC) was accompanied by changes in genes associated with thyroid signalling, growth regulation, and tissue remodelling, including increased expression of tpo and dio3 in the gill, slc5a5 and dio2 in the intestine, and changes in igfals, igfbp7, secisbp2, and thrb-linked pathways in the skin and intestine. In sea bass, 17 °C also produced larger thyroid follicles and greater follicle cell height and larger follicular dimensions compared to other thermal regimes. The thyroid-related transcriptome responses included changes in thrsp and tshba in gill and in igfals, igfbp2a, thrb, spp1, bglap, and matrix-remodelling genes in skin and intestine, consistent with endocrine- dependent tissue remodelling. The results showed that short-term changes in embryonic thermal regimes modified the thyroid in sea bream and sea bass during metamorphosis, and the effect was most pronounced at 17 °C, although the developmental consequences differed. In sea bream, the enhanced thyroid response at 17 °C was associated with remodelling of the gill, skin, and intestine. Sea bass also exhibited an enhanced thyroid response at 17 °C, although the associated remodelling responses varied among tissues, with gill traits most evident at 17 °C and several intestinal and skin characteristics more pronounced at 20 °C. Overall, the data indicate that embryonic thermal history programs species-specific and tissue-specific remodelling responses during metamorphosis, potentially due to a lasting impact on thyroid development and axis activity in two teleosts.

Keywords: Thermal imprinting, Metamorphosis, thyroid, Sea bream, Sea bass, Comparative endocrinology

Acknowledgements: This work was supported by the European Union through the project H2020-SFS2016-2-727610, PerformFISH and Portuguese national funds from FCT through project nº2023.01974.BD, UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020, and CRESC Algarve 2020 and COMPETE 2020, projects EMBRC. PT ALG-01-0145-FEDER-022121 and BIODATA. PT ALG-01-0145-FEDER-022231.

O-067 - Seasonal dynamics of the ovarian follicular cell transcriptome in European sea bass: insights into steroidogenesis and thermal effects

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Introduction: Oocyte development involves systemic hormonal cues and local paracrine signals, between the oocyte and surrounding follicular cells, which coordinate folliculogenesis, steroidogenesis, and provide structural support within the ovary. In European sea bass *(Dicentrarchus labrax)*, a species of major relevance for Mediterranean aquaculture, the molecular pathways governing follicular cell activity are still poorly characterized. In this study, we characterized the transcriptomic dynamics of ovarian follicular cells across the annual reproductive cycle and investigated how elevated temperature modulates steroidogenesis gene expression during vitellogenesis.

Methods: Ovaries sampled across the annual reproductive cycle of adult females were used to isolate follicular cells by enzymatic dissociation, and analyzed by RNA-seq (150 bp PE, Illumina NovaSeq X) to characterize seasonal transcriptomic dynamics

Follicular cells isolated from vitellogenic ovaries were cultured in monolayers at low (15 °C) or high (25 °C) temperatures and stimulated with exogenous steroid precursors: cholesterol, 17α-hydroxyprogesterone, or 4-androstene-3,17-dione. Gene expression and steroid levels were analyzed 24 and 48 hours post-stimulation.

Results & Discussion: Transcriptomic profiling of follicular cells across the annual reproductive cycle revealed marked seasonal changes associated with ovarian development and steroidogenic activity across the four histologically defined stages of oogenesis (previtellogenesis, vitellogenesis, maturation/ovulation, and atresia). The most pronounced transcriptional differences were observed between the end of the spawning period (Spring) and post-spawning/previtellogenesis (Summer), coinciding with follicular atresia and the initiation of new follicle recruitment, suggesting an association with folliculogenesis and ovarian tissue remodeling. A second major transcriptional transition was detected at the onset of vitellogenesis (Autumn) relative to the previtellogenic stage (Summer), whereas comparatively few transcriptional changes were observed during secondary growth, from early vitellogenesis through post-vitellogenesis and maturation.

In parallel, *in vitro* exposure of vitellogenic follicular cells to elevated temperature (25 °C) altered steroidogenic regulation by significantly reducing the expression of cyp19a1, responsible for the final step of estradiol biosynthesis, while aromatase activity was not affected under high-temperature conditions. Together, these findings demonstrate that follicular cell activity in European sea bass is dynamically regulated throughout the reproductive cycle and that elevated temperature can alter ovarian steroidogenesis, with potential implications for reproductive performance under climate change scenarios.

Funding: MCIN/AEI/10.13039/501100011033/ and ERDF a way of making Europe (PID2021-122929OB-C32). ThinkInAzul programme (MICIN/ NextGenerationEU (PRTR-C17. I1)/ GVA-THINKINAZUL/2021/042. A.E. PhD contract (GVA- CIGRIS/2022/002).

O-068 - Photoperiodic sensitivity at puberty in *Oreochromis mossambicus*

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Puberty onset is a pivotal developmental transition in fish, marking the functional activation of the hypothalamic-pituitary- gonadal axis and the beginning of reproductive competence. In teleosts, this process is regulated by complex interactions between neuroendocrine signals and environmental cues such as photoperiod. Despite the ecological and aquacultural importance of Mozambique tilapia (Oreochromis mossambicus), the developmental timeline of puberty and the mechanisms by which photoperiod modulates its onset remain poorly characterised. This study aimed to (i) establish a baseline developmental and histological profile of gonadal maturation under normal photoperiod conditions and (ii) investigate the effects of photoperiod manipulation on gonadal development and brain gene expression during the pubertal window. Under 12L:12D ovaries were dominated by primary oocytes (O1) at 60 dph, the appearance of cortical alveoli stage (O2) by 70 dph, the appearance of early vitellogenic oocytes (O3) at 90 dph and a progressive increase in vitellogenic (O4) oocytes from 100 dph onward, with fully developed oocytes (O5) by 120 dph. Fish subjected to a short-day photoperiod (SNP: 6L:18D) between 70 and 100 days had significantly delayed puberty compared to the NP control (12L:12D) at 100 dph, while those submitted to a long-day photoperiod (LNP: 18L:6D) showed no significant difference from NP. Transcriptomics of brain and pituitary tissue revealed numerous differentially expressed genes between the manipulated photoperiod groups and the control group, with biological functions including photoperiod response, retinoid signalling activation, metabolic and circadian reprogramming, and neuroendocrine regulation. Our results establish that female tilapia become pubertal around 70 dph, as measured by the appearance of oocytes at the cortical alveoli stage, and simultaneously become responsive to inhibitory photoperiod. These findings provide a foundation for future functional studies to establish the endocrine regulation of puberty.

Acknowledgements: This study received Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) to AVMC and fellowship 2023.00917.BD to NJP.

O-069 - Harnessing postbiotics to enhance resilience to microplastic toxicity: insights from gut microbiome and defensome responses in gilthead seabream

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Microplastics pose a significant threat to aquatic organisms due to their widespread presence in marine and freshwater environments. They can be ingested by fish, invertebrates, and other aquatic species, causing physical damage, reduced feeding efficiency, and impaired growth. Moreover, microplastics may act as carriers of toxic chemicals, leading to oxidative stress, reproductive disorders, and other adverse biological effects. Due to the global spread of microplastic pollution and the limited effectiveness of current removal strategies, innovative approaches to mitigate its toxicity are urgently needed. Dietary postbiotics represent a promising alternative, as they can modulate immune responses, support gut barrier function, reduce inflammation, and offer greater stability and safety than conventional probiotics. In this study, juvenile seabream *(Sparus aurata)* were fed for 40 days with a diet enriched with weathered microplastics (MPE), added at 1% of the dry feed weight (MPE group). Microplastics were environmentally weathered for two months at the mouth of the Chienti River, a site characterized by high anthropogenic pressure in the Marche region. To evaluate the ability of postbiotics to counteract MPE- induced toxicity, an additional group received both MPE and a postbiotic consisting of thermally inactivated *Lactobacillus rhamnosus* IMC 501® (MPPE group). A control group and a group receiving the postbiotic alone (P group) were also included. Gut tissues were analyzed using shotgun metagenomics and RNA-seq. Preliminary results revealed significant modulation of transient microbial taxa. In fish exposed to weathered MPE, the abundance of *I. loihiensis* (involved in carbon and nutrient recycling), *C. zancles* (associated with PAH degradation), and *A. muciniphila* (linked to mucus turnover and gut barrier integrity) decreased. Fish in the MPPE group showed similar reductions in *A. muciniphila* and *E. hormaechei* abundance, alongside an increase in P. oxalicum, suggesting enhanced fungal activity that may improve the degradation of dietary polysaccharides and contribute to nutrient turnover within the gut environment. The increase in *C. amphilecti* observed in the P group further suggests enhanced carbon utilization. Differential gene expression analysis revealed distinct defensome- related responses among treatments. The MPE group exhibited transcriptional changes consistent with cellular stress, indicating activation of antioxidant and detoxification pathways typically associated with adaptation to toxic challenges. In contrast, the P group showed modulation of genes involved in redox homeostasis, glutathione metabolism, and cytoprotective mechanisms, supporting a beneficial adaptive response compatible with hormetic activation of the defensome. Notably, the MPPE group displayed a markedly attenuated defensome signature compared with the MPE group, suggesting that postbiotic supplementation mitigated MPE-induced cellular stress and contributed to the restoration of redox balance. Overall, these findings demonstrate that dietary postbiotics can effectively counteract the detrimental effects of microplastic exposure by promoting protective physiological responses, enhancing host resilience, and restoring redox homeostasis. Through the modulation of gut microbial communities and defensome-related pathways, postbiotics emerge as a promising, sustainable, and practical tool to mitigate microplastic-

induced toxicity, opening new perspectives for improving fish health, welfare, and environmental sustainability in aquaculture and natural ecosystems facing increasing microplastic contamination.

Funded by the Italian Ministry of Research (MUR) (NEOPLASTIC) 2022WAJ28Y and European Commission under grant agreement No 101131121, AQUASERV project

O-070 - Integrated physiological and genomic responses of juvenile chub mackerel (*Scomber japonicus*) to acute hypoosmotic stress

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Salinity fluctuations are becoming increasingly frequent in coastal marine environments as a consequence of climate change and represent a major physiological challenge for marine teleosts. Osmoregulation under hypoosmotic conditions requires coordinated endocrine and cellular responses; however, the mechanisms underlying acute low-salinity tolerance in pelagic marine fishes remain poorly understood. In this study, we investigated integrated physiological, branchial, transcriptional, and genomic responses to acute hypoosmotic stress in juvenile chub mackerel *(Scomber japonicus)*, a commercially important marine species with growing aquaculture potential.

Fish were exposed to a stepwise reduction in salinity from 35 ppt to 6 ppt, followed by gradual recovery to seawater conditions. Survival, behavioral responses, gill histology, expression of key branchial osmoregulatory genes, and genome- wide single nucleotide polymorphism (SNP) variation were evaluated throughout the experiment. Juvenile *S. japonicus* tolerated salinity reduction to 10 ppt without mortality, although feeding activity and general behavior were markedly suppressed. In contrast, exposure to 6 ppt resulted in severe behavioral impairment and approximately 50% mortality, indicating a critical threshold for acute hypoosmotic tolerance.

Histological observations indicated changes in branchial morphology under severe hypoosmotic stress, although further quantitative evaluation is required to characterize these alterations. Transcriptional analyses demonstrated coordinated salinity-dependent regulation of major osmoregulatory genes, including aquaporin 3 (*aqp3*), Na^+/K^+-ATPase α1 (*atp1a*), solute carrier family 12 (*slc12a*), and cystic fibrosis transmembrane conductance regulator (*cftr*), with expression patterns partially recovering following return to seawater. These findings indicate substantial physiological plasticity of the branchial ionoregulatory system during acute salinity challenge.

To investigate the genetic basis of variation in salinity tolerance, genome-wide SNP analysis using Genotyping by Random Amplicon Sequencing-Direct (GRAS-Di) identified multiple loci associated with survival under low-salinity exposure. Candidate genes included oxidation resistance 1 (*oxr1a*), chloride voltage-gated channel 3 (*clcn3*), solute carrier family 26 member 2 (*slc26a2*), potassium voltage-gated channel subfamily A regulatory beta subunit 1a (*kcnab1a*), Na^+/K^+ transporting ATPase interacting 4 (*nkain4*), and sodium voltage-gated channel alpha subunit 8a (*scn8aa*), suggesting roles for oxidative stress defense, ion transport regulation, membrane homeostasis, and neuroendocrine signaling pathways in hypoosmotic adaptation. Functional enrichment analyses further highlighted biological processes related to epithelial transport, ion regulation, and cellular stress responses.

Together, these results demonstrate that acute hypoosmotic tolerance in juvenile *S. japonicus* is mediated by integrated behavioral, branchial, transcriptional, and polygenic responses that support ion and water balance under osmotic stress. This study provides new insights into the physiological and genetic mechanisms underlying salinity adaptation in marine teleosts and contributes to our understanding of endocrine-regulated osmoregulatory processes in changing aquatic environments.

O-071 - Endocrine regulation of nutrition, metabolism and fat storage in a changing world

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This overview will summarise endocrine mechanisms of vertebrate nutrition at different levels of the brain-pituitary- glandular axis. Special focus is laid on fat metabolism and storage, which are relevant also for reproduction and offspring. Adipose tissue is a prerequisite as energy deposit and essential for physiological processes such as reproduction and supply to the offspring. Nevertheless, it has also emerged as an accumulation site for endocrine-disrupting compounds in the modern environment, particularly for aquatic and arctic mammals. Adipose tissue has also been recognised as a source of proinflammatory cytokines. Current concepts of obesity under investigation in the pathomechanisms of global health issues such as cancer, diabetes, cardiovascular diseases are presented.

Plenary Lecture — PL5 (Aula Magna)

Endocrine system under chemical pressure: cross-species and 3D human models in a One Health framework

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The accelerating production and environmental dissemination of synthetic chemicals, including pesticides, plasticizers, and persistent industrial compounds, has placed the endocrine system under increasing pressure. Within a One Health framework, endocrine disruption represents a shared biological vulnerability linking ecosystem, animal, and human health. Studies integrating aquatic vertebrate models with advanced human 3D systems show that contaminants of emerging concern target conserved pathways regulating reproduction, metabolism, development, and skeletal homeostasis.

Research in fish demonstrates that widely used agrochemicals and plastic-derived compounds, at environmentally relevant concentrations, exert biological effects. Chronic exposure of fish to glyphosate alters reproductive physiology in both sexes, affecting germ cell differentiation, steroidogenesis, gonadal structure, and epigenetic programming, while disrupting metabolic and hormonal pathways essential for reproductive competence. Similarly, bisphenols and phthalates impair reproductive and metabolic functions and frequently exhibit non-monotonic dose–response relationships that challenge conventional toxicological paradigms.

A key mechanistic hub underlying these responses is the endocannabinoid system (ECS), a conserved lipid signaling network integrating reproductive, metabolic, neuroendocrine, and skeletal functions. Chemical interference with ECS signaling is associated with alterations in energy metabolism, appetite regulation, bone remodeling, gonadal activity, and fertility, often in a sex- and context-dependent manner. In addition, several contaminants induce persistent epigenetic modifications in reproductive genes, suggesting that their effects may extend beyond directly exposed individuals.

The growing diversity of emerging contaminants, including per- and polyfluoroalkyl substances (PFAS), further expands the spectrum of biological targets. Human osteoblast and intestinal organoid models reveal impacts on skeletal development, extracellular matrix organization, oxidative balance, and genome stability, supporting the translational relevance of experimental findings obtained in aquatic organisms.

Importantly, the microbiota is emerging as both a target and a modulator of chemical toxicity. Probiotic interventions effectively mitigate gonadal, intestinal, skeletal, and genotoxic alterations *in vivo* and/or in human 3D systems, enhancing resilience to contaminant exposure. Together, these findings identify shared mechanisms of vulnerability across species and support microbiota-targeted interventions as promising tools to reduce the health burden of environmental contaminants within a One Health perspective.

SOA11 - Multifactorial control of fish reproduction by the secretograninergic system

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A multitude of neuropeptides regulate pituitary gonadotropin release and spawning in teleosts. The conspicuous lack of effects of gonadotropin-releasing hormone (GnRH) gene knockout (KO) in adult zebrafish has accelerated the search for other key regulators. Among these, the secretogranin-2 (Scg2) derived peptide secretoneurin (SN) is emerging as a critical regulator of GnRH, LH and ovulation. Scg2 is evolutionarily conserved from cartilaginous fishes to mammals and exists as duplicated paralogs in teleosts, *scg2a* and *scg2b.* Proteolytic processing of these precursors generates the peptides SNa and SNb, with SNa representing the ancestral form homologous to mammalian SN. Single and double *scg2a/scg2b* frameshift mutant zebrafish display marked behavioural and reproductive deficits. Through exosomal release from Scg2a neurons, SNa stimulates the proliferation and excitability of GnRH3 neurons in zebrafish. SN also stimulates LH production and release directly at the pituitary. Injection of SNa leads to ovulation within 6 hr. We have developed new zebrafish models with the CRISPR/Cas9-mediated complete excision of the coding region of *scg2a* and *scg2b*. Within line breeding has established that spawning and fecundity are reduced in *scg2a-/-, scg2b-/-* single and double knockout (DKO) fish compared with their sibling WT, and single KO groups exhibited delayed oviposition and low quiver behaviour. Ovarian histology showed reduced oocyte maturation at stages 4 and 5 in DKO fish. Proteomic analyses revealed extensive remodelling of both pituitary and ovarian tissues in DKO females. The pituitary exhibited downregulation of core secretory machinery, metabolic pathways, and cytoskeletal processes, while the ovary showed disrupted secretion, vesicle trafficking, signalling, and marked reductions in extracellular matrix and focal adhesion proteins. These findings highlight the roles of Scg2/SN in zebrafish reproduction, affecting fecundity, oocyte maturation, and hormone regulation within the HPG axis, underscoring its role in fertility. The authors acknowledge funding from NSERC-Canada, the Chinese Academy of Sciences, and the contributions of D. Peng and C. Lu.

O-072 - Transcriptomic and neuropeptidomic insights into nervous system regulation of shell biomineralization in the mediterranean mussel (*Mytilus galloprovincialis)*

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Marine bivalves produce highly structured biomineralized shells whose growth is controlled by the mantle, a richly innervated secretory tissue. While a biomineralization toolbox containing shell matrix proteins (SMP), ion transporters, and enzymes has been extensively catalogued, the upstream regulatory mechanisms, and in particular the contribution of the central nervous system (CNS), remain largely unknown. Our previous work demonstrated that neuropeptides, including the calcitonin (CALC) system, regulate mantle function and shell repair in the Mediterranean mussel *Mytilus galloprovincialis*, and that severing the cerebropleural ganglia (CPG) commissure blocks shell regeneration. Herein an integrated transcriptomic and neuropeptidomic analysis reveals the molecular structure of CPG - mantle signaling and uncovers a novel regulatory system mediated by long non-coding RNAs (lncRNAs). A shell damage-repair assay was established in *M. galloprovincialis* by sanding both shell valves. Three experimental groups were compared: controls (natural), sanded-only, and sanded with CPG commissure severed. RNA-Seq libraries were generated from the right and left mantle of each individual (24 h post-treatment) and differential gene expression was analyzed by DESeq2 and co-expression network analysis (WGCNA). The analysis combined a map of the distribution of nerve fibers in the mantle under different conditions using immunohistochemistry (anti-5-HT) with the results of neuropeptide data analysis of the mantle. Transcriptomic profiling revealed that the right and left mantles of M. galloprovincialis display asymmetric gene expression patterns even under baseline conditions, suggesting lateralized regulation of shell formation. CPG commissure severing significantly modified the mantle transcriptome, with 81 differentially expressed genes (DEGs) identified relative to sanded controls, of which a disproportionately high fraction were lncRNAs - a class not previously implicated in the bivalve biomineralization toolbox. None of the 81 DEGs corresponded to typical SMPs, indicating that the CPG modulates shell formation primarily through regulatory rather than structural gene networks. Immunohistochemical analysis revealed a significant change in the density and distribution of serotonergic (5-HT) fibers at the mantle edge after the CPG was severed. During shell repair, the peptidome of the mantle tissue identified locally secreted neuropeptides, in addition to the calcitonin system, including APGW amide peptides, thus expanding the known range of neuropeptides acting on the mantle. Overall, these findings establish a model in which the bivalve CNS acts as a master regulator of biomineralization via a neuroendocrine relay: CPG-derived neuropeptides, transmitted through the nerve, coordinate lncRNA-mediated gene regulatory networks in the mantle to control shell deposition.

Acknowledgements

This study received Portuguese national funds from FCT through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020. ZL was supported by a EUREMAP postdoctoral fellowship.

O-073 - Functional diversification of GLWamide-GPCR system in the cnidarian *Nematostella vectensis*

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Neuropeptide signaling represents one of the earliest forms of intercellular communication in animals, but few peptide receptors are known to be functionally characterized outside bilaterians. In cnidarians, it has been shown that GLWamide peptides represent a highly conserved neuropeptide family involved in muscle contraction, metamorphosis, and larval settlement. However, GLWamide cognate receptors remained largely unknown.

Here, we identify and functionally characterize G protein–coupled receptors (GPCRs) activated by GLWamide and GLWamide related peptides in the sea anemone *Nematostella vectensis*. Using a bioinformatic approach, we identified eight class A GPCRs and their neuropeptide agonists. Receptor deorphanization and pharmacological profiling were achieved by heterologous expression in HEK293 cells combined with reporter gene assays. Our dose-response analyses showed that almost all characterized receptors respond to GLWamide-family peptides with marked differences in ligand selectivity, potency, and some of them are dependent on peptide amidation. Some receptors were broadly activated, while others were highly specific for certain GLWamide peptides, which indicates functional diversification within the signaling system.

To explore the cellular organization of these signaling systems, we mapped GPCR and neuropeptide expression into single- cell transcriptomic datasets from developmental and adult stages of the sea anemone *Nematostella vectensis.* The differential expression analyses revealed stage-specific expansion and diversification of the peptidergic signaling networks. GLWamide-related ligands and receptors were enriched primarily in neuronal and neuroglandular cell populations with several GPCRs co-localizing with ligand-expressing neuronal clusters, supporting muscle-associated tissues. Reconstruction of peptidergic connectomes revealed highly interconnected signaling networks in adult animals, with neuronal subsets acting as major signaling hubs and non-neuronal tissues such as retractor cells and mucosal glands functioning as major targets. Comparative analyses showed that developmental stages possess less integrated signaling architectures, suggesting that peptidergic networks increase in complexity during maturation.

Together, our results show that GLWamide signaling in cnidarians is both evolutionarily conserved but also functionally diversified. By integrating receptor pharmacology with single cell connectomics in Nematostella vectensis, this study provides insight into how ancient neuropeptidergic systems are spatially organized and integrated across tissues during early animal evolution.

O-074 - Neuropeptides and their potential roles in scleractinian corals

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Neuropeptides act as modulators and hormones, and play important roles in various biological processes in animals. The presence and functions of neuropeptides in corals are still largely unknown. To have a better understanding of the physiological functions of neuropeptides in stony corals, we established a transcriptome of the stony coral Fimbriaphyllia ancora, and explored the presence and potential roles of neuropeptides. By transcriptome and 5' and 3' RACE-PCR, we successfully elucidated the full-length of Gly-Leu-Try-amide family (GLWamide) and Glu-Gly-Arg-Phe-amide family (QGRFamide) neuropeptides precursor cDNA in F. ancora. The deduced amino acid sequences possessed 7 repeats of GLW motifs in GLW precursor and 17 repeats of QGRF motifs in QGRFamide. Tissue distribution of GLWamide and QGRFamide precursors by QPCR analysis revealed that precursor transcripts were highly expressed in the mouth and tentacle in the polyp. Immunohistochemical staining demonstrated that GLWamide- and QGRFamide-positives were mainly distributed in mouth and tentacle and less in gonads and mesenterial filament. Furthermore, *in vitro* treatments of the isolated mouths and tentacles with both neuropeptides showed to induce the deformation and contraction of these isolated tissues. These results strongly suggested that GLWamide and QGRFamide are involved in muscle activity of coral polyp. These neuropeptides caused the tentacle contraction may be related to coral behaviors including reproduction, feeding, stress, or others. Furthermore, for GnRH/corazonin superfamily, immunoreactive and biological active gonadotropin-releasing hormone (irGnRH-like) peptides by radioimmunoassay were also detected and peaked in coral during the spawning. Coral extracts and mGnRH agonist have a similar dose dependent effect on the release of luteinizing hormone (LH) in the black porgy fish pituitary cells (*in vitro* culture); while mGnRH receptor antagonist specifically blocked the LH stimulatory release of coral extracts. Corazonin-like precursor cDNA was cloned and its expression was higher in the testis during the spawning compared to non-spawning period. The involvement of GnRH-like/corazonin superfamily in the coral reproduction is suggested. Our studies shed light on the presence, origin, and evolution of neuropeptides in invertebrates, and also their roles in basal metazoa.

O-075 - Evidence of family B1 GPCR genes in Cnidaria rewrites their evolutionary history

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The G protein-coupled receptors (GPCRs) of Family B1 are a functionally diverse group of neuropeptide receptors that are activated by peptides and regulate key physiological processes across Metazoa. In vertebrates, five major Family B1 receptor subfamilies have been described and roles assigned in development, metabolism, stress regulation, and neuroendocrine signalling. The Family B1 receptors in invertebrates are much less explored compared to the vertebrates but the studies that exist indicate there are a much broader diversity of receptor genes including the existence of lineage- specific subfamilies. Herein, we investigated the origin and diversification of Family B1 receptor subfamilies in invertebrates. Using comprehensive comparative genomics we studied Family B1 receptor gene evolution across a broad range of metazoan lineages (non-bilaterians, protostomes, and deuterostomes) and based on the results we established a unified evolutionary framework for the classification of Family B1 GPCRs across Metazoa. Thirty-nine (39) well annotated invertebrate genomes representing different Phyla were searched using a Family B1 HMM model and the retrieved receptor sequences were annotated and compared to the vertebrate homologues. We confirmed that members of Family B1 GPCRs are present in protostome and deuterostome genomes, and contrary to the results of previous studies, receptor gene homologues were identified in an early metazoan lineage, the Cnidaria (jelly fish, corals), although they were absent from Porifera (sponge) genomes. In cnidarians, Family B1 GPCR genes were found in 24 species belonging to different classes and gene number per species was variable and, in the hydroid, Hydra vulgaris, the multiple receptor genes identified mapped in tandem suggesting they resulted from species-specific duplication events. The cnidarian deduced receptor proteins retained the conserved signature motifs of a typical Family B1 receptor and CLANS, and phylogenetic analysis revealed that they formed a separate cluster outside of the other bilaterian receptor subfamily clusters. The present comprehensive analysis of the Family B1 GPCRs in metazoa provides new insights into the early evolutionary history of peptide signalling systems and suggests that the origin of Family B1 GPCRs predates the divergence of major eumetazoan lineages. The current evolutionary model of Family B1 receptors during bilaterian evolution will be reconsidered taking into consideration the results of the cnidarian and hydroid genes and considering the lineage-specific gene duplications, losses, and the emergence of distinct receptor subfamilies in the protostomes and deuterostomes.

Acknowledgments: This study received Portuguese national funds from FCT - Foundation for Science and Technology (FCT), I.P., and the European Regional Development Fund (ERDF) through UID/04326/2025 (DOI https://doi.org/10.54499/UID/04326/2025), UID/PRR/04326/2025 (DOI https://doi.org/10.54499/UID/PRR/04326/2025) and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020), and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through contracts EMBRC.PT ALG-01-0145-FEDER-022121 and BIODATA.PT ALG-01-0145-FEDER-022231.

O-076 - Insulin signalling and ecdysis in the German cockroach

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The insulin/insulin-like growth signaling (IIS) pathway plays a central role in regulating multiple essential physiological processes in animals. The identification of three insulin receptors (InRs) in the cockroach *Blattella germanica* (BgInR1, BgInR2, and BgInR3) prompted us to investigate their specific contributions to the regulation of distinct physiological events. Previous studies from our group demonstrated that BgInR2 is the paralog that retains the predominant activity in reproductive functions. Then, we focused on elucidating the roles of the three *B. germanica* InRs in molting and their involvement in the hormonal regulation of the different phases of this process.

Our analyses revealed that, as in reproduction, BgInR2 is the InR playing the predominant role in molting, consistent with its highest expression levels in tissues and organs associated with this process. Functional assays revealed that RNAi-triggered depletion of BgInR2 impairs nymphal growth and dysregulates ecdysteroid synthesis in the prothoracic gland. These alterations lead to delayed and reduced production of ecdysis triggering hormone (ETH), ultimately resulting in a postponed and defective ecdysis. These findings show that IIS, through BgInR2, regulates and coordinates ecdysteroid production, thereby coupling molting to growth.

O-077 - Insect neuropeptides influence the electrophysiological properties of mammalian excitable cells

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Neuropeptides are key regulators of physiological processes across animal phyla, acting as neurotransmitters, neuromodulators, and neurohormones. They modulate neural and non-neural tissues by altering membrane bioelectrical properties and intracellular signaling pathways. Despite the rapid identification of novel insect neuropeptides, their potential interactions with mammalian systems remain poorly understood. In particular, it is unclear whether structural homology between insect and mammalian neuropeptides translates into functional cross-reactivity at the receptor level.

In this study, we investigated the effects of selected neuropeptides on the electrophysiological activity of mammalian cells (cardiomyocytes and neurons). Extracellular action potentials (EAPs), including their amplitude and duration, were recorded using a 6-well multielectrode array system following application of synthetic peptides. The tested compounds included insect neuropeptides with established myotropic activity (proctolin, crustacean cardioactive peptide – CCAP, myosuppressin – MS, and periviscerokinin – PVK) as well as mammalian neuropeptides (neuropeptide FF, RFamide-related peptide 1, and neuromedin U). Contractile activity was assessed by videomicroscopy, and peptide cytotoxicity was evaluated in HeLa cells.

Peptides were analyzed in two experimental concentrations, 10-8 and 10-6 M. Tested neurohormones modulated electrophysiological parameters in a dose-dependent manner, with a distinct activity profile. CCAP exerted the strongest effect on spike frequency, whereas RFRP1 significantly altered spike amplitude.

These findings demonstrate that insect neuropeptides can modulate mammalian cell electrophysiology, supporting the concept of functional conservation and potential cross-reactivity between phylogenetically distant peptidergic systems.

The study was supported by the National Science Center, project no. 2021/41/B/NZ3/00221.

O-078 - The mystery of myoglianin: a key regulator of development in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae)

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Myoglianin (Myo), a member of the TGF-β superfamily, is known to regulate several important physiological and developmental processes in insects, including juvenile hormone signaling, ecdysteroid biosynthesis, growth, metamorphosis, reproductive development and lifespan [1-3]. Previous studies in different insect species demonstrated that Myo signaling affects developmental timing, body growth, and the transition from juvenile to adult stages through interactions with endocrine pathways [4]. However, the role of this gene in the desert locust, *Schistocerca gregaria*, remains largely unexplored. The present work provides experimental evidence that silencing Myoglianin using RNA interference strongly affects nymphal development and adult reproduction in the desert locust. Knockdown of Myo delayed molting, reduced growth, and caused developmental abnormalities including wing deformities. Most notably, Myo silencing induced supernumerary molting into an additional sixth nymphal instar, indicating disruption of normal metamorphosis. In adults, Myo knockdown also delayed female reproductive maturation and reduced ovary development. So, in the current study, the effects of Myoglianin knockdown on developmental timing, metamorphosis, somatic growth, wing formation, and reproductive physiology were examined in the desert locust, a very devastating migratory pest species [5]. The significant conclusions of this study provide new insight into the conserved regulatory role of Myoglianin in insect development and may contribute to the future development of species-specific and environmentally sustainable locust pest control strategies.

Keywords: Hormone, Insect, Metamorphosis, Reproduction, RNA interference, TGF-β

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Acknowledgements:

The authors would like to sincerely thank Evelien Herinckx, Arnold Van Den Eynde, Lore Smekens, and Marijke Christians for their valuable technical support with locust rearing and laboratory maintenance. The authors also greatly appreciate the assistance of the PhD student Stijn Goossens for his help in carrying out the experiments. The authors further acknowledge the financial support provided by the KU Leuven Research Foundation (C14/24/076).

SOA12 - The zebrafish pineal gland reclaims the spotlight

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Temporal organization of behavioral and physiological processes is governed by an endogenous timing mechanism, the circadian clock. The organization of the circadian system differs fundamentally between mammals and non-mammalian vertebrates. In mammals, circadian rhythm coordination and photoreception are performed by distinct autonomous structures in the hypothalamic suprachiasmatic nucleus (SCN), the “master clock”, and the retina, respectively. A well- studied example of SCN-driven rhythm is the rhythmic melatonin secretion from the pineal gland. In contrast, in non- mammalian vertebrates the pineal gland is photoreceptive and can drive melatonin rhythms independently and, in some species, the pineal gland is even considered to act as the master oscillator. Nevertheless, non-mammalian vertebrates possess a more distributed circadian system in which reciprocal interactions among retinal, pineal, and hypothalamic oscillators, contribute to circadian regulation. The relative contribution of these oscillators to circadian rhythms of behavior varies among species.

Zebrafish represent an extreme example of a decentralized circadian system. In addition to the eyes and the pineal gland and the presence of deep-brain photoreceptors, virtually all peripheral tissues are directly light responsive, allowing their circadian clocks to be entrained independently by environmental light. Despite this unique feature of zebrafish peripheral clocks, the pineal gland remains a key circadian pacemaker, and growing evidence suggests that its role in regulating behavior extends beyond circadian rhythms. Nevertheless, the widespread photoreceptive capacity of peripheral tissues underscores the need to better define the organization of the zebrafish circadian system and to clarify the specific contribution of the pineal gland within this distributed network. This presentation will highlight recent advances from our laboratory and others that provide new insights into the mechanisms and functions of the zebrafish pineal gland and will provide evidence for its role both as an illumination detector and as a central circadian pacemaker.

O-079 - Evolution of light-regulated cell signalling pathways in vertebrates

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Fish biology is very much shaped by sunlight. Direct light exposure triggers changes in the expression of genes as well as miRNAs that are functionally connected with the circadian clock, DNA damage repair, mitochondrial function and heme metabolism. Furthermore, light has a considerable effect on neuroendocrine function. This central role played by light raises many questions concerning how light is detected by, and triggers responses in fish cells. ROS, MAPK signalling as well as the D-box promoter enhancer element play a central role in directing light inducible gene expression in fish. D-box binding transcription factors belonging to the PARbZip family represent convergence points for regulation by light, UV and ROS. We have documented fundamental changes in light dependent signalling cascades as well as photoreceptive proteins in mammals as well as in species of blind cavefish that have evolved for millions of years in complete isolation from sunlight. Using a comparative study involving zebrafish, blind cavefish and mouse cell lines, we reveal how the PARbZip transcription factor TEF, is regulated by ROS and light via phosphorylation by the stress MAP Kinases, jnk and p38. Changes in these phosphorylation sites in cavefish and mammalian TEF account for loss of regulation by ROS and light in these species. Our results reveal that sunlight-driven signalling pathways and gene expression control mechanisms which are also responsive to oxidative stress represent key targets for evolutionary adaptation in vertebrates.

O-080 - A model to study conserved food-reward circuits: ghrelin and dopamine during food anticipatory activity in goldfish

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Food intake is not solely determined by the organism's energetic requirements but is strongly shaped by hedonic and motivational mechanisms. Within this framework, food anticipatory activity (FAA), a circadian increase in locomotor activity preceding a predictable meal, has been proposed as a behavioural indicator of an anticipatory arousal state associated with food expectation. In goldfish (*Carassius auratus*), this motivational state is characterized by anxiety-like responses, like increased thigmotaxis (the preference for staying close to walls, avoiding open zones) and scototaxis (the natural aversion to bright white areas in favour of dark ones). Ghrelin, a key orexigenic hormone, modulates FAA and these behavioural states in goldfish. Moreover, this hormone in mammals regulates dopaminergic signalling within the mesolimbic reward system through the ventral tegmental area (VTA) and nucleus accumbens. In teleosts, however, the functional conservation of this pathway remains unclear, as dopaminergic neurons are located in the posterior tuberculum (PT) rather than in the mesencephalon. Therefore, the aim of this study was twofold: first, to investigate whether dopamine mediates ghrelin- induced anxiety-like states during food anticipatory activity (FAA) in goldfish; and second, to explore the possible involvement of a PT–accumbens-like dopaminergic pathway underlying these effects. We evaluated the influence of dopamine on anxiety-like behaviour with two approaches. First, during FAA fish received intraperitoneal (IP) injections of D1-type (SCH23390; 1 µg/g body weight, bw) or D2-type (sulpiride; 2 µg/g bw) dopamine receptor antagonists. Second, at 2–3 h postprandial (when endogenous ghrelin is low) anxiety-like behaviour was assessed after IP injection of ghrelin (10 pmol/g bw) alone or combined with each dopamine antagonist. Exogenous ghrelin reproduced the FAA anxiety-like state, increasing thigmotaxis in the open field test and scototaxis in the black–white test. Blockade of D1-like receptors partially attenuated these responses, whereas D2 antagonism completely reversed the anxious state both during FAA and after ghrelin injection, indicating a predominant role of D2-like receptors in mediating the motivational and affective components of FAA. To characterize the regional distribution of dopamine receptor subtypes, brain regions (olfactory bulb, rest of telencephalon, hypothalamus, rest of diencephalon, and rhombencephalon), were collected from euthanized goldfish and analysed via qPCR. The expression profile revealed a structured pattern, with a predominance of D2-like receptors in telencephalic and diencephalic regions linked to motivational processing. Finally, to study ghrelin's effects on dopamine release in the accumbens-like region and the activation of the PT, fish received an IP injection of vehicle or ghrelin at 2 h postprandial and telencephalic accumbens-like and diencephalic PT region were dissected under a stereo microscope. Neurochemical analyses showed that ghrelin increased dopamine and DOPAC levels in the telencephalic accumbens-like region, supporting the recruitment of a reward-related pathway. Additionally, ghrelin upregulated *c-fos*, *tyrosine hydroxylase*, and *ghrelin receptor* expression in the PT. Together, these results demonstrate that ghrelin recruits a dopaminergic pathway to modulate the anxious state observed during FAA in goldfish, with D2-like receptors playing

a central modulatory role. The integration of behavioural, molecular, and neurochemical data suggests the existence of a teleost ghrelin–PT-dopamine axis functionally analogous to the mammalian ghrelin–VTA–dopamine circuit. This evolutionary conservation highlights a fundamental mechanism through which metabolic signals interact with reward processing and affective-like states to regulate feeding behaviour, validating FAA as a robust model for studying the motivational components of feeding.

O-081 - Effects of free temperature choice on clock genes, neurogenesis and brain monoaminergic function in Atlantic salmon parr

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Atlantic salmon production systems are commonly characterised by barren tanks and cages that provide limited sensory and behavioural stimulation, potentially constraining the expression of rewarding natural behaviours and impairing welfare. Consequently, environmental enrichment strategies have emerged as promising approaches to improve fish welfare by increasing environmental complexity. Such enrichments may enhance behavioural flexibility, promote cognitive engagement, and allow fish to express their individual preferences, all of which are considered important contributors to positive welfare states. As ectothermic vertebrates, fish rely on behavioural thermoregulation and actively select thermal environments according to their physiological demands and internal affective states. Thermal preference is closely linked to neuroendocrine regulation. In this study, Atlantic salmon parr *(Salmo salar)* were provided with a thermal gradient to investigate daily rhythms of thermal preference and the neurophysiological mechanisms potentially associated with this behavioural choice. We compared fish maintained under thermal choice conditions with controls kept at constant temperature by analysing 24-hour profiles of brain monoamines and the mRNA expression of genes associated with circadian control, neuroplasticity and neuronal activation. Our results demonstrated that salmon parr displayed a robust daily pattern of thermal selection, preferring warmer areas during daylight hours and cooler temperatures at night. These behavioural rhythms were accompanied by temporal changes in clock genes and brain monoaminergic activity. In addition, exposure to thermal gradient enhanced the expression of genes related to neurogenesis, suggesting increased neuronal plasticity and cognitive stimulation. This work was funded by a TNA from AQUAEXCEL 3.0 (PID: 33003).

O-082 - Circadian regulation of extracellular vesicles

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The mammalian circadian system is composed of a hierarchical multi-oscillator structure, which can regulate numerous physiological processes to exhibit 24-hour rhythmic oscillations, enabling the organism to adapt the environmental conditions. Extracellular vesicles (EVs) encompass a heterogeneous collection of secreted membrane-encapsulated vesicles that can signal between cells and tissues. To date, there only exists initial published evidence of circadian regulation of EV cargos *in vitro* and sparse data referring to the oscillation of the number of EVs. To investigate whether the circadian clock regulates EV number and cargo, we employed various *in vitro* cultured cell lines, such as C2C12-dervived myotubes and AML12 hepatocytes. Supernatants were collected at different time points after synchronization, and EVs were purified using size-exclusion chromatography (SEC) followed by determination of EV number by flow cytometry and analysis of protein contents by Western blot. Our preliminary evidence suggests that EV release from synchronized C2C12 myotubes exhibits a circadian rhythm. Furthermore, Western blot analysis revealed that circadian clock proteins are detected in EVs from the supernatants of both AML12 and C2C12 cells. These initial results indicate that the number of EVs released from muscle exhibits rhythmic oscillation, and provides the first evidence that EVs can transport circadian clock proteins, suggesting that EVs may play an important role in inter-cellular and inter-organ circadian communication.

O-083 - Sex and day/night dependent regulation of muscle and liver proteome

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Molecular clocks and sex differences are important regulators of metabolism in peripheral tissues, but their impact on tissue and secreted protein abundance is unclear. The liver and skeletal muscle are key metabolic organs, acting as endocrine organs through the release of secreted proteins including hepatokines and myokines and other secreted factors involved in intercellular and inter-organ communication.

Here, we analyzed liver and skeletal muscle tissue proteomes using an ex vivo approach, in which tissues were incubated in chemically defined medium continuously bubbled with carbogen for 45 min at 37°C then the media containing secreted proteins was concentrated ∼20-fold with 3 kDa cutoff centrifugal filters for downstream DIA LC-MS/MS proteomic analysis of both tissue and secreted fractions under day (ZT8)/night (ZT20) and female/male conditions. Our results show clear differences between tissue and secreted proteomes in both organs, with sex being the main source of variation and a subset of proteins showing day/night-dependent regulation. We also identify proteins shared between liver and muscle secretome, supporting the hypothesis that these tissues may participate in coordinated inter-organ communication.

Understanding how sex and day/night cycles shape liver and muscle secretomes may provide insight into inter-organ communication pathways relevant to, obesity, insulin resistance, type 2 diabetes and MASLD (fatty liver disease).

O-084 - Behavioural effects of dopamine depletion induced by CRISPR targeting of tyrosine hydroxylase in zebrafish

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Dopamine is a critical neurotransmitter of the vertebrate encephalon involved in motor function and diverse behavioural activities, including motivational/reward processes, aggression, sexual behaviour, learning and memory. Dysfunction of central dopaminergic systems is linked to severe neurological disorders such as Parkinson's disease (PD) and attention deficit and hyperactivity disorder (ADHD), schizophrenia and addiction, all associated with decreased synaptic dopamine levels and/or sensitisation of dopaminergic systems.

Tyrosine hydroxylase (Th) is the rate-limiting step in the first stage of catecholamine synthesis, promoting the conversion of tyrosine into L-dopa that is subsequently processed into dopamine. This neurotransmitter is further processed into norepinephrine and epinephrine. Due to the additional genome duplication of teleost fish (TSGD), the zebrafish genome carries two paralogue genes, *th1* and *th2*, with differential distribution in the encephalon. *th1* is much more widely expressed, whereas *th2* expression remains confined to three populations localised in the rostral preoptic area, posterior tubercle and caudal hypothalamus. We designed CRISPR probes to knockout both th version in zebrafish. A stable *th2* mutant line harbouring a CRISPR-induced 2-bp deletion in the first exon was generated through successive inbreeding. *th1*- targeted CRISPR induced different indels also in the first exon, but embryos were never viable. To validate the *th2* knockout phenotype, brain dopamine levels were quantified by HPLC, the number of TH-positive cells in the posterior hypothalamus was assessed by immunohistochemistry, and potential compensatory changes in *th1* and *th2* expression were evaluated by qPCR in whole-brain samples from *th2*$^{-/-}$ and *th2*$^{+/+}$ zebrafish. Although dopamine levels in whole-encephalon extracts were comparable between mutant and wild-type (WT) fish, the number of Th-immunoreactive cells in the caudal hypothalamus was significantly reduced in the mutants. The behavioural phenotype of mutant fish was characterised using a battery of assays, including open field, novel object, novel tank, and social preference tests. In addition, circadian locomotor activity was evaluated in both mutant and wild-type fish. In all behavioural tests, total distance moved, mean swimming velocity, and angular velocity did not differ significantly between WT and *th2*$^{-/-}$ fish, suggesting that motor function was not impaired in the mutants. Similarly, total circadian locomotor activity was comparable between genotypes. However, *th2*$^{-/-}$ fish appeared to exhibit reduced locomotor activity in the upper region of the tank, a behaviour commonly associated with increased anxiety-like responses. This interpretation was consistently supported by the results of the other behavioural assays, collectively indicating an anxiety-like phenotype in *th2*$^{-/-}$ fish. The social preference index, measured as the percentage of time close to the shoal, did not differ between phenotypes, although stimulus exploration, calculated as the ratio between time spent visiting shoal or empty areas of the tank and total experimental time, was remarkably elevated in mutant fish, thus suggesting an exploratory hyperactivity that could partially resemble the phenotype observed in ADHD patients. In summary, these findings suggest that disruption of *th2* expression promotes an anxiety-like phenotype while enhancing exploratory behaviour in zebrafish, without significantly affecting motor function.

POSTERS

Posters presented during Poster Session 1 (Monday, September 7, 17:00–18:00, Hall) and Poster Session 2 (Wednesday, September 9, 17:00–18:30, Hall), grouped below by topic following the order of the official Poster Index.

1. Invertebrate neurohormones governing physiology and behavior: a symposium honoring the careers of Angela Lange and Ian Orchard

P-001 - Deorphanisation and evolutionary analysis of monoamine GPCRs in the sea urchin *Mespilia globulus*

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As basal invertebrate deuterostomes, echinoderms occupy a pivotal phylogenetic position, being more closely related to chordates than other commonly used invertebrate models, and thus provide a unique perspective on the evolutionary trajectory of signalling systems from invertebrates to vertebrates. This project investigates the organisation and function of monoaminergic G-protein coupled receptors (GPCRs) in the sea urchin *Mespilia globulus*, a genetically tractable echinoderm with a recently characterised genome.

While biogenic amines like dopamine and norepinephrine are universal physiological regulators, the evolutionary boundaries between invertebrate-specific pathways (such as octopamine and tyramine) and vertebrate-like networks remain poorly defined in intermediate deuterostome lineages. In insects, octopamine and tyramine are often considered functional homologues of vertebrate adrenergic signalling, yet it is unknown whether echinoderms employ similar, vertebrate-like, or mixed monoaminergic receptor repertoires.

Given the unique evolutionary placement of echinoderms, we hypothesize that they represent a critical evolutionary stage where these receptor families co-existed. To test this, we used an integrated approach combining in silico sequence mining, Cluster Analysis of Sequences (CLANS), molecular cloning, and receptor deorphanisation assays, we identified candidate rhodopsin-family monoamine GPCRs from the *M. globulus* genome. Our bioinformatic screening successfully identified candidate GPCRs alongside core biosynthetic enzymes, including aromatic L-amino acid decarboxylase (AADC)-like, tyrosine hydroxylase/tryptophan hydroxylase (TH/TPH)-like, and dopamine beta-hydroxylase (DBH)-like sequences, suggesting the presence of baseline machinery for monoamine production in this species. We successfully cloned 17 candidate receptor transcripts and completed initial bioluminescence ligand screening for 14 expressed candidates against a panel of 20 monoamine-related compounds.

Deorphanisation assays revealed that several of these receptors are activated by several different monoamines, demonstrating the presence of functional monoaminergic GPCR signalling in this species. The candidate receptor Mg_278553 responded strongly to both dopamine and octopamine, representing direct functional cross-reactivity between catecholamine and phenolamine pathways within a single receptor. Another candidate, Mg_142181, displayed broad sensitivity across multiple biogenic amines, which may reflect a promiscuous, unspecialized ancestral binding pocket.

Additionally, Mg_259860 showed a clear and selective activation by dopamine. Ongoing work focuses on dose–response analyses to quantify ligand specificity and signalling potency, allowing us to determine which monoamine pathways are likely to be physiologically relevant.

Our results provide a functional framework for understanding how echinoderms utilise biogenic amines for cellular communication. By defining the monoaminergic receptor landscape of *M. globulus*, this work establishes this species as a tractable model for investigating monoaminergic signalling in an invertebrate deuterostome. In doing so, it sheds light on both ancestral and derived features of deuterostome monoaminergic systems and provides new insight into the evolution of neurotransmitter signalling across animals.

P-002 - Evidence for Juvenile hormone regulation of cellular and humoral immunity during vitellogenesis in the classical insect model *Rhodnius prolixus*

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The study of physiological processes influencing insect propagation is essential for the development of control strategies aimed at limiting the spread of species harmful to humans without disrupting ecological balance. In insects, both immunity and reproduction are critical processes that directly impact survival and population expansion. *Rhodnius prolixus*, a major vector of Chagas disease in Latin America, has historically served as an important model for studies of insect physiology and endocrinology. Juvenile hormone (JH), an invertebrate-specific hormone, plays a central role in insect development and reproduction, but its contribution to immune regulation during reproduction remains poorly understood. This project aims to investigate the role of JH in the innate immune response of adult female R. prolixus during reproduction. Our preliminary findings indicate that vitellogenesis is associated with a shift in immune investment, characterized by reduced humoral immune responses together with enhanced cellular immunity. Considering that JH titers rise markedly during vitellogenesis, we hypothesized that this hormone participates in coordinating this immune modulation. To address this hypothesis, we combined molecular approaches (RT-qPCR), *in vitro* assays involving JH incubation, and microscopy-based analyses, including yeast challenge experiments and calcium imaging using Cal-520 in hemocytes. Preliminary results revealed activation of calcium signaling in hemocytes following JH exposure *in vitro*, suggesting a direct role of the hormone in regulating cellular immune functions. In parallel, evidence from *in vitro* fat body incubation assays suggests that JH also modulates pathways associated with humoral immunity. Together, these findings support a role for JH as a key regulator coordinating reproductive physiology and immune function during vitellogenesis in *R. prolixus*, providing novel insights into the mechanisms underlying reproduction–immunity interactions in an important disease vector.

P-003 - An *in vivo* effect of recombinant salmonid insulin-like growth factor binding protein-1a

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Insulin-like growth factor (IGF)-1 is a 7.5-kDa polypeptide structurally similar to proinsulin and has the mitogenic action as well as hypoglycemic action. Circulating IGF-1 plays an important role in growth regulation by mediating action of growth hormone (GH) and suppressing GH secretion from the pituitary gland as a negative feedback loop. The activity of circulating IGF-1, however, is regulated by multiple IGF-binding proteins (IGFBPs). Six IGFBPs are present in mammals and they are capable of inhibiting or/and promoting the action of IGFs depending on the type, post-translational modification and cellular environment. IGFBP-1 is one of major circulating forms and generally inhibits IGF action by preventing it from interacting with the receptors. Teleosts have two co-orthologs of each type except IGFBP-4 due to a third round of whole-genome duplication (WGD) and salmonids experienced an extra round of WGD and retained 22 copies of igfbp genes, suggesting adaptive advantage of tune-tuning IGF-1 actions. However, function of each IGFBP subtype is poorly understood in salmonids.

We have been producing recombinant salmonid IGFBPs using bacterial expression system for functional analysis and antibody generation. Their activity has been examined *in vitro* using primary salmonid pituitary cell culture, where effect of addition of recombinant IGFBP in combination with IGF-1 on GH release is assessed. On the other hand, evaluating *in vivo* effect of IGFBP on the IGF-mediated growth is challenging due to a large quantity of recombinant protein required for growth study and the difficulty in optimizing the experimental conditions.

The present study produced recombinant rainbow trout (rt) IGFBP-1a and examined an acute *in vivo* effect on the insulin-like activity of IGF-1. rtIGFBP was expressed with fusion partners thioredoxin and His/S-tags (Trx.His.S.) using pET-32a expression vector and isolated from bacterial proteins by Ni-affinity chromatography. A milligram order of Trx.His.S.rtIGFBP-1a with IGF-binding ability was obtained and used for injection study to modulate the hypoglycemic action of IGF-1. Juvenile rainbow trout were injected with recombinant human (rh) IGF-1 (25 pmol/g body weight) with or without Trx.His.S.rtIGFBP-1a (50 pmol/g) into the dorsal muscle and collected for blood. An injection of rhIGF-1 resulted in a reduction of plasma glucose levels in 4 h whereas a co-injection with Trx.His.S.rtIGFBP-1a restored plasma glucose levels. In a second experiment, plasma glucose levels in fish injected with rhIGF-1 and Trx.His.S.rtIGFBP-1a were unexpectedly increased 4 h after injection but returned to the basal level at 24 h. A third experiment confirmed that Trx.His.S.rtIGFBP-1a suppressed the hypoglycemic effect of rhIGF-1 8 h after injection. These data show that recombinant trout IGFBP-1a inhibits the short-term effect of IGF-1 *in vivo*. This system allows a quick screening of the activity of a recombinant IGFBP prior to conducting a longer-term growth study.

This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENIHI Grant Numbers 25H00936.

P-004 - Effect of recombinant rainbow trout insulin-like growth factor binding protein-1a on cultured trout muscle cells

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Insulin-like growth factor (IGF) exerts their mitogenic effect through endocrine, paracrine and autocrine pathways. Regardless of the mode of action, the activity of IGFs is regulated by multiple IGF-binding proteins (IGFBPs). Circulating IGFBPs are responsible for delivering IGFs to target tissues or sequestering them from the circulation. In salmonid circulation, at least three IGFBPs are consistently detected and they have been identified as IGFBP-1a, -1b and -2b. Analysis using a primary pituitary cell culture suggests that both salmonid IGFBP-1a and -1b are inhibitors of IGF action. However, effects on other tissues have not been assessed. In salmonid muscle, *igfbp-1a* mRNA is consistently detected while *igfbp-1b* is hardly detected. Thus, IGFBP-1a could affect the availability of endocrine and local IGFs to muscle cells. The present study produced recombinant rainbow trout IGFBP-1a and examined its effect on cultured trout myocytes.

Recombinant trout (rt) IGFBP-1a was expressed with fusion partners thioredoxin and His/S-tags (Trx.His.S.) using pET-32a expression vector. Expressed recombinant protein in the insoluble fraction was solubilized by urea and isolated from bacterial proteins by Ni-affinity chromatography. Trx.His.S.rtIGFBP-1a was refolded by dialysis. The fusion partner in Trx.His.S.rtIGFBP-1a was cleaved by enterokinase K and rtIGFBP-1a was purified by using reversed-phase HPLC. Juvenile rainbow trout of 4–10 g were used for primary muscle culture. Stem cells were isolated from white muscle by enzymatic dissociation with collagenase type IA and trypsin, seeded onto poly-L-lysine/laminin coated plates, and cultured at 18 ºC for 2 days in growth medium (DMEM supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic). Recombinant human (rh) IGF-1 or -2, with or without rtIGFBP-1a, was added to the cultures. Treatments effects, including cell proliferation assessed using the Click-iT EdU Imaginig Kit, are currently being analyzed.

P-005 - Endosomal microautophagy (eMI): an unknown player in the regulation of growth and metabolism?

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Autophagy (literally meaning "self-eating") is a process that encompasses different mechanisms by which cells deliver cytoplasmic constituents to the lysosome for degradation. Among these pathways, two selective mechanisms stand out: chaperone-mediated autophagy (CMA) and endosomal microautophagy (eMI). Their specificity is based on the recognition of KFERQ and KFERQ-related pentapeptide motifs, present in several metabolic enzymes, by the chaperone HSC70. In contrast to CMA, which targets substrates to lysosomes via the receptor LAMP2A, eMI substrate internalization depends on the ESCRT machinery. eMI substrates may then be degraded within the late endosome (LE) or upon fusion with a lysosome. Beyond proteolysis, the selective degradation of key proteins allows the modulation of cellular functions.

Of major interest, it has been recently described that eMI substrates can also follow an alternative route leading to secretion. Whereas the biological effects of the degradation of KFERQ-bearing proteins are known, the physiological implications of their extracellular release remain unexplored. Interestingly, in silico analyses have revealed that the sequence of growth hormone (GH) —the main growth regulator in vertebrates, including fish— from several Mediterranean aquaculture species contains at least one conserved putative KFERQ-related motif. This suggests that fish GHs are potential substrates for CMA and eMI degradation and, potentially, candidates for eMI-mediated secretion. Thus, eMI could be involved in rerouting GH toward degradation or secretion, and its modulation has great potential to boost fish growth.

In this regard, our recent research has identified —for the first time in fish— an eMI-like process in rainbow trout hepatocytes that is activated by specific stimuli, including oxidative stress, high glucose, and nutrient deprivation, among others. To better understand the modulation and physiological functions of eMI in trout, we are currently analyzing how amino acids regulate eMI activity and which signaling pathways are involved. Future studies will focus on exploring the role of eMI in the secretion of GH and other growth factors to expand our knowledge of eMI's contribution to the control of fish growth and metabolism.

This study has received support from the grant RYC2023-045487-I funded by MICIU/AEI/10.13039/501100011033 and by ESF+, and the projects PID2024-161074NA-I00, PID2024-161415OB-I00, and PID2023-149563OB-I00, funded by MICIU/AEI/10.13039/501100011033 and by ERDF/EU.

P-006 - Insights into cellular signalling cascade elicited by taurine in cultured myotubes from European sea bass (*Dicentrarchus labrax)*

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European sea bass (*Dicentrarchus labrax*) is one of the most commercially valuable species in the Mediterranean aquaculture. However, the use of highly substituted diets for this species is hampered due to its highly carnivorous habit. The non-protein amino acid taurine has been previously reported as a potential dietary supplement in fish aquaculture, enhancing both growth and antioxidant capacity. Here, to better understand the signalling response triggered by taurine in muscle, proteomic, phosphoproteomic and protein turnover (pulsed-SILAC) analyses were carried out on cultured myotubes (Day 12-myoblasts in 23ºC in 2.5% CO2) from European sea bass.

Taurine treatment (10mM for 24h) showed higher protein turnover and protein phosphorylation, suggesting an increase in protein synthesis capacity and a higher proliferation rate (e.g., Pcna). Proteins with decreased expression upon taurine treatment were enriched in processes related to cell development, while protein involved in translation processes were upregulated. Taurine also increased the phosphorylation of several members of the mTOR signalling pathway, such as Akt, Map4k3, Srrm1, as well as transcriptional factors involved in protein synthesis (e.g., Eif3a), or myogenic-related binding protein (e.g., Paxbp1). These findings support a proteinogenic role of taurine in muscle cells by enhancing mTOR signalling and ribosomal activity, suggesting a switch towards a more proliferative status (hyperplasia) modulating muscle development.

P-007 - Transcriptomic insights into two gilthead sea bream (*Sparus aurata*) genetic lines fed with organic diets

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Organic aquaculture, an alternative to conventional aquaculture, requires feeds that meet strict standards for either fish quality, animal welfare and regulatory compliance. The current scenario shows a restrictive expansion due to scarce availability of certificed feed ingredients, the ban on using synthetic amino acids, and strict organic rules. Therefore not only shall alternative ingredients be explored but also new genotyped / phenotyped cultured animals. Here, in the following study, two Gilthead sea bream (*Sparus aurata*) genetic lines (fast-growing and standard) coming from Cromaris (Croatia) were fed with different organic and conventional diets (designed by INRAE, Legouessant Aquaculture and Lallemand SAS). One novel organic diet featuring partial fishmeal replacement with hydrolysed yeast extract and inclusion of organic poultry meal, while the other organic diet was a commercial one without yeast extract.

Three isoproteic (43%) and isolipidic (17%) diets (conventional (CC), commercial organic (CO), novel organic (NO)) were tested in a 3 × 2 factorial design across eighteen 800-L tanks in a controlled RAS. Fish (133.5 ± 0.25 g) were PIT-tagged, reared under optimal conditions over 94 days. Clear differences were seen between diets and genetic lines, being the fast- growht the weightenest one. Samples from muscle, liver and bone were processed in order to shed light into the differences between genetic lines in regard to their transcriptome. Despite observing differences because of genetic factor, fish fed with novel organic diet showed a higher expression on muscle proteolytic genes such as trim63 and fbxo32, and an upregulation in myod1 (first steps of myogenic development) suggesting differences in muscle remodelling. Together with assessing the GH/Igf pathway and the upcoming results of bone and liver expression will help to understand the response of these two genetic lines to organic diets.

P-008 - Inhibition of growth hormone receptor (GHR) activation by pegvisomant exacerbates neuroinflammation and motor deficits following neonatal hypoxia

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Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are essential regulators of neonatal growth, metabolism, and brain development. Perinatal hypoxia disrupts the somatotropic axis and contributes to long-term neurological impairments. Although GH has been proposed as a neuroprotective factor, the role of endogenous GH signaling in the response to neonatal hypoxic injury remains incompletely understood. Therefore, this study evaluated the effects of GH treatment and pharmacological GH receptor (GHR) blockade using the antagonist pegvisomant on metabolic, endocrine, inflammatory, and behavioral outcomes following neonatal hypoxia. Wistar rat pups received pegvisomant (5 mg/kg, s.c.); three doses were administered at 48-h intervals beginning on postnatal day 1 (P1) to inhibit GH receptor signaling before hypoxic injury. Global hypoxia was induced at P2 by exposure of animals to 8% O_2 for 2 h. Following hypoxia, a separate group received bovine GH (0.1 mg/kg/day, s.c.) for five consecutive days (P2–P6). Blood glucose concentrations were measured 3 h after hypoxia, and serum IGF-1 levels were determined at P6. Long-term effects of the treatments were evaluated in adulthood (P64) by qPCR analysis of pro-inflammatory cytokines and components of the GH/IGF-1 system. Motor coordination was assessed using the static rod and rotarod tests. Hypoxia reduced blood glucose concentrations compared with those of GH- treated animals under both normoxic and hypoxic conditions, while pegvisomant significantly reduced blood glucose in both control and hypoxic neonatal rats. At P6, hypoxia decreased circulating IGF-1 levels compared with vehicle-treated controls, whereas GH treatment increased IGF-1 concentrations relative to hypoxic animals. In contrast, pegvisomant treatment reduced circulating IGF-1 levels under both normoxic and hypoxic conditions. In adulthood, neonatal GH receptor blockade increased the expression of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in both males and females compared with hypoxic animals that did not receive the antagonist. Furthermore, pegvisomant induced upregulation of GH, IGF-1, and their receptor transcripts, suggesting activation of compensatory mechanisms within the somatotropic axis. Behavioral analyses demonstrated that hypoxia-induced motor deficits persisted into adulthood in both male and female rats. However, GH treatment improved performance in the static rod and rotarod tests in comparison with hypoxic animals, whereas GH receptor blockade with pegvisomant exacerbated motor impairment under both normoxic and hypoxic conditions. These findings indicate that endogenous GH signaling contributes to metabolic homeostasis and protection against the long-term consequences of neonatal hypoxia. Early inhibition of GHR activation aggravates neuroinflammatory responses and motor dysfunction while inducing compensatory activation of the GH/IGF-1 system. Together, these results support a neuroprotective role for GH during early brain development and suggest that disruption of GH signaling increases vulnerability to the adverse long-term effects of neonatal hypoxic injury. Acknowledgments: G. Courtois, N. Hernández, E. de los Ríos, S. Pech, D. Gasca, A. González, R. Martínez, M. García, M. Carbajo, and A. Castilla INB-UNAM, for technical support. This work was supported by grants from PAPIIT-DGAPA-UNAM (IN207524, IN209124, IN218325).

P-009 - Establishment and characterization of two monoclonal bone- derived cell lines from gilthead sea bream (*Sparus aurata*) as *in vitro* models for bone biology research

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Bone-derived primary cell cultures are valuable *in vitro* models for studying fish skeletal physiology; however, their inherent heterogeneity may compromise experimental reproducibility and limit their use in long-term functional studies. Therefore, the establishment of monoclonal cell lines provides more stable and standardized models for investigating skeletal development and the endocrine role of bone in teleost fish. This study aimed to establish two monoclonal bone-derived cell lines from gilthead sea bream (*Sparus aurata*), and to evaluate their proliferative capacity, differentiation potential, transfection efficiency, and molecular profile.

For this purpose, primary cultures were established from vertebrae of gilthead sea bream. Monoclonal cell lines were obtained by limiting dilution of passage 30 primary cells, subsequent expansion, and serial passaging. The resulting cell lines, designated SaBone-D7 and SaBone-G5, were successfully maintained in culture for more than 50 passages and remained viable after multiple cryopreservation. Cell viability was evaluated using the MTT assay. Cells were seeded at varying densities: 500, 1000, 2000, 4000, 6000, 8000, and 10000 cells/well in a 96-well plate to determine optimal seeding conditions. To assess their capacity to sustain growth under reduced serum, cells were cultured under different fetal bovine serum (FBS) concentrations: 0%, 2%, 5%, and 10%. In addition, population doubling time was calculated from direct cell counts obtained over a 96-h culture period to evaluate proliferative capacity. Transfection efficiency was evaluated by electroporation using fluorescent reporter plasmids. Finally, osteogenic and adipogenic differentiation potential was assessed through specific staining, and the expression of bone-related genes was analyzed by real-time quantitative PCR.

Both SaBone-D7 and SaBone-G5 cell lines displayed stable growth *in vitro* but distinct morphology under bright-field microscopy. Indeed, SaBone-D7 cells appeared larger than SaBone-G5 cells under routine culture conditions. This difference was confirmed by flow-cytometry-based cell sorting, suggesting phenotypic differences between the two monoclonal populations. Cell viability analyses revealed that a seeding density of 12.500 cells/cm^2 (i.e., 4×10^3 cells/well) results in the highest viability after 48h in both cell lines, whereas after 96h, optimal viability was at 8×10^3 cells/well for SaBone-D7, and 6×10^3 cells/well for SaBone-G5. Nevertheless, higher cell densities did not improve the viability in either cell line. In contrast to the primary culture, which exhibited increased viability with increasing serum concentration, both SaBone-D7 and SaBone-G5 cells showed similar viability profiles in media containing 5% and 10% FBS, indicating a lower dependence on serum supplementation. Population analyses revealed high proliferative capacity in both cell lines, with doubling times <24h. Furthermore, both cell lines retained the ability to undergo matrix mineralization under differentiation conditions, indicating the preservation of osteogenic properties after long-term culture (i.e., 15 days). A high transfection efficiency was achieved in both cell lines in preliminary experiments, highlighting their suitability for gene function and molecular studies. Quantitative analyses regarding potential adipogenic differentiation and gene expression are currently in progress.

Overall, the rapid growth, long-term stability, maintenance of osteogenic mineralization capacity, and high transfection efficiency of SaBone-D7 and SaBone-G5 highlight their potential as robust *in vitro* models for functional, molecular, and endocrine studies in fish, reducing the dependence on primary cultures and animal use in research.

Funded by project PID2024-161415OB-I00 (MICIU/AEI/10.13039/501100011033 and ERDF/EU). IR predoctoral fellowship PRE2021-100391 (MICIU/AEI/10.13039/501100011033 and ESF+). DGS is a Serra-Húnter Associate Professor.

P-010 - GH/IGF expression in liver and muscle of European sea bass fingerlings under dietary and exercise treatments

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Improving fish growth in aquaculture remains a major challenge which must be addressed together with the need for more sustainable production strategies. In this context, we are exploring nutritional and physiological conditioning strategies during the fingerling stage in European sea bass (*Dicentrarchus labrax*) with the aim of obtaining juveniles with enhanced growth and resilience. In this context, we focused on the role of GH/IGF axis and related molecules which are fundamental in the regulation of muscle development.

E. sea bass fingerlings were fed for two months with four experimental diets differing in fishmeal inclusion and functional additive supplementation: high fishmeal (HFM), low fishmeal (LFM), low fishmeal plus antioxidants (LFM-AO), and low fishmeal plus taurine (LFM-TAU). Each diet was tested under voluntary swimming (VS) or sustained swimming exercise (SS; 1.5 BL s^{-1}), yielding a total of eight experimental groups. At the end of the trial, fish were sacrificed, and biometric indexes and tissue samples were collected for gene expression and histological analyses. Although biometric indexes did not differ significantly among groups, sustained swiming fish and fish fed either HFM or LFM-TAU showed the best growth performance.

Hepatic GH/IGF axis gene expression showed increased *ghr2* levels in fish fed the low fish meal without additives, whereas *ghr1* remained unaffected. The expression of *igf1* and *igf2* was higher in fish subjected to sustained swimming, with a similar trend observed for their receptors. In skeletal muscle, *igf2*, *igfbp6* and *pax7* were upregulated in fish fed the high fishmeal diet, but *igfbp5* showed higher expression in fish fed with low fishmeal without additives.

In conclusion, these preliminary data indicate that diet and exercise differentially modulate the GH/IGF axis in both liver and skeletal muscle. However, liver showed a stronger response to both diet and exercise, whereas skeletal muscle was mainly influenced by diet. Further analyses will help to clarify the role of these pathways in growth regulation in European sea bass fingerlings.

Supported by PID2023-149563OB-I00 funded by 2021SGR-00713, Generalitat de Catalunya, MICIU/AEI/10.13039/501100011033 FEDER/UE [IR1] and by EUAqua.org. R.I., S.R. and M.M. are predoctoral fellows PRE2021-100391, Grant No 101181589, PREP2023-001685, respectively.

P-011 - Autocrine mRNA and miRNA effects of IGF-I and IGF-II treatments in IGF of cultured muscle satellite cells in European sea bass (*Dicentrarchus labrax*)

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Skeletal muscle represents a major proportion of fish body mass and is one of the most relevant tissues for aquaculture production. In teleosts, muscle growth can continue after sexual maturation through persistent myogenesis, involving both hyperplasia and hypertrophy, and is regulated by signalling pathways including insulin-like growth factors I and II (IGF-I and IGF-II). This study aimed to investigate the role of IGF-I and IGF-II under optimal proliferative conditions by analysing genes and miRNAs associated with myogenesis, the GH/IGF axis and the mTOR pathway in European sea bass *(Dicentrarchus labrax)* primary myocyte cultures.

Primary myocyte cultures were established from epaxial muscle of European sea bass fingerlings. Cells were treated with chicken IGF-I, sea bream IGF-I or chicken IGF-II at different concentrations, together with serum free and serum supplemented controls. Cell proliferation was assessed by BrdU incorporation, and selected treatments were subsequently used for gene and miRNA expression analyses during the late proliferation and differentiation stages.

BrdU assays showed increased proliferation in cells treated with chicken IGF-I and sea bream IGF-I at 100 ng/mL, whereas chicken IGF-II required 500 ng/mL to induce a similar response. These results suggest that lower concentrations of IGF-I are sufficient to promote myocyte proliferation in European sea bass. In addition, igf1 expression remained low at both culture stages, whereas igf2 showed higher expression levels, supporting a predominant role of IGF-I during early proliferative responses and a greater involvement of IGF-II during differentiation. No significant differences were detected in the expression of myogenic, growth-related or signalling pathway genes among treatments, although expression patterns differed between culture stages, reflecting the transition from proliferation to differentiation. miRNA expression profiles also varied between stages, suggesting their potential involvement in myogenic progression.

Overall, these preliminary results suggest a differential role of IGF-I and IGF-II during European sea bass myogenesis, with IGF-I promoting proliferative responses at lower concentrations and IGF-II being more associated with later differentiation stages, while miRNAs may contribute to the regulation of myogenic progression.

Supported by PID2023-149563OB-I00 funded by 2021SGR-00713, Generalitat de Catalunya, MICIU/AEI/10.13039/501100011033 and FEDER/UE. M.M. is a predoctoral fellow PREP2023-001685.

P-012 - Divergent roles of Cck receptors in digestion, reproduction, lipid metabolism, and growth in medaka

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Cholecystokinin (Cck) is a multifunctional peptide hormone that regulates digestion, appetite, and reproduction in vertebrates. Although three *cck receptor* subtypes have been identified in teleost fish, the distinct physiological roles of each receptor subtype remain poorly understood, and additional functions beyond classical Cck signalling may have been overlooked. In this study, we generated medaka (*Oryzias latipes*) knockout lines for three distinct Cck receptors (*cck1r*$^{-/-}$, *cck2ra*$^{-/-}$, and *cck2rb*$^{-/-}$) using CRISPR/Cas9 system and conducted comprehensive phenotypic, biochemical, and transcriptomic analyses to elucidate their physiological functions.

The *cck1r*$^{-/-}$ fish exhibited markedly reduced digestive enzyme secretion, including trypsin, chymotrypsin, lipase, and amylase, accompanied by reduced feed efficiency and significant growth retardation during early development. These results provide direct evidence that Cck1r plays a critical role in digestive enzyme regulation and early somatic growth in teleosts.

In contrast, the *cck2rb*$^{-/-}$ fish displayed pronounced phenotypic alterations, including complete female infertility, delayed ovarian development, reduced plasma vitellogenin levels, and significant lipid accumulation in the whole body, liver, and muscle. These fish also showed enhanced somatic growth and improved feed efficiency during the post-juvenile stage. Gene expression analysis revealed decreased pituitary *fshb* expression and increased hepatic *igf1* expression, suggesting disruption of reproductive endocrine signaling and activation of somatic growth pathways. Histological observations confirmed immature ovaries and steatosis-like hepatic lipid accumulation. Transcriptomic analysis further indicated substantial modulation of lipid metabolism pathways, including upregulation of genes related to lipid transport and Ppar signaling, and downregulation of cholesterol catabolic processes.

The *cck2ra*$^{-/-}$ fish showed comparatively mild phenotypic changes, including moderate lipid accumulation and slight reductions in reproductive performance, suggesting a secondary or modulatory role in the regulation of lipid metabolism and reproductive maturation. Collectively, these findings demonstrate clear functional divergence among Cck receptor subtypes in teleosts: Cck1r primarily mediates digestive function and early growth, Cck2rb acts as a central regulator integrating lipid metabolism, reproduction, and somatic growth, and Cck2ra plays a supportive role in metabolic and reproductive processes. These results extend the classical understanding of Cck signaling beyond digestion and appetite regulation, revealing its involvement in lipid metabolism and growth control. This study provides new insights into endocrine–metabolic integration in vertebrates and represents the first comprehensive functional characterization of all three Cck receptor subtypes within a single vertebrate model. These findings may also contribute to improving metabolic regulation and feed efficiency in aquaculture species.

P-013 - Effects of peripheral cannabinoid receptor 1 blockade on hepatic metabolism in rainbow trout

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This study investigated the role of the peripheral endocannabinoid system (ECS) in the regulation of hepatic metabolism in rainbow trout (*Oncorhynchus mykiss*). The ECS is known to regulate energy balance in mammals through cannabinoid receptors, especially CB1, which influence glucose, lipid, and protein metabolism in peripheral tissues such as the liver. Although ECS involvement in appetite regulation has been studied in fish, its role in peripheral metabolic regulation remains poorly understood. Therefore, the objective of this work was to evaluate the effects of the peripheral CB1 receptor antagonist AM6545 on hepatic metabolism under different nutritional conditions.

Juvenile rainbow trout were maintained under controlled laboratory conditions and divided into three nutritional groups: fasted (FA), fed (FE), and refed (RE). Fish received an intraperitoneal administration of either AM6545 or vehicle control using a coconut oil-based implant that enabled sustained release over five days. Plasma and liver samples were collected to analyze metabolite concentrations, enzymatic activities, mRNA abundance by RT-qPCR, and AMPK protein levels by Western blot. Nutritional status strongly influenced metabolic parameters. Fasted fish showed lower plasma glucose and triglyceride levels, depleted hepatic glycogen reserves, and increased activities of enzymes related to gluconeogenesis (PEPCK, FBPase), glycogen breakdown (GPase), lipid oxidation (CPT-1), and amino acid catabolism (GDH). Refeeding generally restored metabolic values toward those observed in fed fish. mRNA abundance of the energy sensor AMPKα1 (*prkaa1*) was also higher in fasted fish, consistent with activation under low-energy conditions. Peripheral CB1 blockade with AM6545 produced several metabolic effects, particularly under fasting conditions. In plasma, antagonist-treated fish exhibited lower fatty acid levels, suggesting reduced peripheral lipogenesis. In the liver, the antagonist significantly modified activities of enzymes involved in carbohydrate and lipid metabolism. Thus, fasted antagonist-treated fish showed reduced hexokinase (HK), phosphoenolpyruvate carboxykinase (PEPCK), fatty acid synthase (FAS), and ATP-citrate lyase (ACLY) activities compared to untreated controls, indicating reduced glycolytic, gluconeogenic, and lipogenic capacities. Conversely, glutamate dehydrogenase (GDH) activity increased markedly in fasted antagonist-treated fish, suggesting enhanced amino acid catabolism. mRNA abundance analyses revealed more limited effects of the antagonist. AM6545 increased the mRNA abundance of genes related to glycogen synthesis (*gys1*) and glucose metabolism (*gck*, *g6pc2b*), while decreasing fbp1 expression in some nutritional conditions. Expression of ECS-degrading enzymes (*magl1* and *faah*) increased during fasting, supporting a relationship between ECS activity and energy status. However, no significant changes were detected in hepatic AMPK phosphorylation or total AMPK protein abundance, suggesting that the peripheral ECS may not strongly regulate these pathways at the protein level in trout liver.

Overall, this study suggest that the peripheral ECS could participate in the regulation of hepatic metabolism in rainbow trout, mainly affecting enzymatic activities associated with lipid and carbohydrate metabolism. The effects of CB1 blockade were most evident during fasting, highlighting an interaction between ECS signaling and nutritional status. These findings provide new evidence that the peripheral endocannabinoid system (ECS) may play a role in regulating hepatic metabolism in teleost fish

P-014 - Ghrelin induces while leptin decreases metabolic rate in goldfish

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Energy balance in vertebrates depends on the coordinated integration of energy intake and expenditure through neuroendocrine signals informing central pathways involved in feeding, metabolism, stress responses, and behavioural activation. In teleost fish, the orexigenic role of ghrelin is relatively conserved, promoting feed intake and, at least in goldfish, also enhancing food anticipatory activity and anxiety-like responses. Physiological leptin effects appear less conserved and more variable among species and nutritional conditions, although in general it is considered an anorectic signal and participates in lipid metabolism. Despite the recognized role of both hormones in energy balance regulation, their influence on metabolic output in fish remains poorly understood. This represents a necessary step to deepen our understanding of why teleost show highly heterogeneous responses to these hormones (especially to leptin) and to clarify how both ghrelin and leptin signalling may be adaptively modulated depending on environmental and physiological conditions.

Thus, the aim of this study was to investigate how ghrelin and leptin influence metabolic rate in goldfish (Carassius auratus). To this end, oxygen consumption rate (MO2) of individual goldfish was measured by intermittent flow respirometry. Each animal was placed individually in a 362.29 mL respirometer chamber under the same environmental conditions as their acclimation tanks (12L:12D and 22 ºC) with water flowing inside the chambers (300 L h−1). Goldfish were intraperitoneally injected with different treatments (mentioned below) at 10:00 h, after a 24-h fasting, and immediately placed into the respirometry chambers in which metabolic rate was continuously monitored for 24 h.

In the first procedure, fish received vehicle (teleost saline, 10 mL/g body weight, bw), ghrelin (10 pmol/g bw), the ghrelin receptor antagonist D-Lys (100 pmol/g bw), or the combined treatment. In the second, fish were treated with vehicle (10 mL/g bw) or leptin (1 µg/g bw). Data were analysed for the 24-h period and separately for the photophase and scotophase.

Across all experiments, goldfish exhibited a robust diurnal rhythm in oxygen consumption, characterized by higher levels during the photophase and transient peaks immediately after chamber introduction and at light transitions. Importantly, none of the pharmacological treatments altered this endogenous rhythmicity, showing that hormones act without disrupting circadian organization.

More importantly, ghrelin significantly increased metabolic rate, especially during the photophase. This ghrelin-induced increase in MO2 was reversed by its antagonist (D-Lys) confirming a specific effect through ghrelin receptors. In contrast, leptin-treated fish showed a significant reduction in oxygen uptake compared with controls, an effect maintained throughout the 24 h, demonstrating that leptin robustly suppresses metabolic rate at both photophase and scotophase. Our data suggest that the functional divergence of ghrelin and especially leptin signalling in teleosts may be partly explained by differences in the regulation of energy expenditure relative to mammals, in which leptin generally increases metabolic rate whereas ghrelin decreases it.

Overall, these findings provide evidence that ghrelin enhances energy expenditure while leptin decreases it in goldfish, at least under our experimental conditions. This highlights the critical role of

peripheral signals in metabolic regulation and offers relevant insights into the evolution of neuroendocrine networks coordinating energy balance and feeding behaviour in fish.

Acknowledgements:

Project PID2022-136288OB-C32 funded by MCIN/AEI /10.13039/501100011033

P-015 - PCB-126 impairs intracellular and metabolic signalling in rainbow trout intestinal cells

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Feedborne pollutants increasingly threaten fish intestinal health by disrupting nutrient sensing, energy metabolism, and barrier function. Among them, 3,3',4,4',5-pentachlorobiphenyl (PCB-126), the most potent dioxin-like polychlorinated biphenyl congener, represents a significant threat to wild fish populations because it persists, bioaccumulates, and biomagnifies along aquatic food webs, and it also poses risks to aquaculture due to its occurrence in fishmeal-based aquafeeds. Although the adverse effects of PCB-126 on fish growth and performance have been documented, the molecular mechanisms underlying its toxicity remain poorly understood. This study evaluated the impact of PCB-126 on metabolic and intracellular signalling pathways in the rainbow trout (*Oncorhynchus mykiss*) intestine using epithelial cell lines derived from the proximal (RTpiMI) and distal (RTdiMI) intestine.

Cells were seeded onto 12-well plates and exposed to three concentrations (1 nM, 100 nM and 1 µM) of PCB-126 in serum- deprived L-15 medium for 2 and 24 h. Following exposure, total RNA was extracted, and the relative abundance of genes involved in intracellular calcium signalling (G protein subunit alpha i-gnai, phospholipase C beta 1-*plcb1*, phospholipase C beta 3-*plcb3*, inositol 1,4,5-trisphosphate receptor type 1-*itpr1*, inositol 1,4,5-trisphosphate receptor type 3-*itpr3*), energy sensing (AMP-activated protein kinase catalytic subunit alpha 2-*Ampkα2/prkaa2*, mechanistic target of rapamycin-*mtor*, sirtuin 1–*sirt1*), and nutrient (particularly fatty acid) sensing (cluster of differentiation 36-*cd36*, fatty acid transport protein 4- Fatp4/slc27a4, G protein-coupled receptor 84-*gpr84*, monocarboxylate transporter 1a-*Mct1A/slc16a1*, free fatty acid receptor *2b2a-ffar2b2a*) was quantified by qPCR.

Major results showed that PCB-126 induced a consistent activation of the PLC–IP_3–Ca^{2+} axis in cells from both intestinal regions, particularly at 24 h, as evidenced by enhanced *plcb1* and *itpr1/itpr3*. Concurrently, gnai downregulation suggests reduced inhibitory Gi signaling, supporting stronger Ca^{2+}-dependent responses under stress. PCB-126 also modulated energy-sensing pathways, with mtor downregulation in RTdiMI and sirt1 upregulation in RTpiMI being consistent, supporting a shift toward energy-saving and stress-resistance responses. PCB-126 also promoted early (2 h) increases in lipid uptake genes (*cd36, slc27a4, slc16a1*), particularly in RTpiMI, followed by later (24 h) normalization or repression, pointing to a transient enhancement of fatty acid handling that may contribute to the metabolic phenotype described for PCBs.

Overall, findings demonstrate that PCB-126 exerts toxic mechanisms that disrupt endocrine and metabolic signalling pathways in rainbow trout intestinal cells. These results highlight the intestinal epithelium as a key endocrine–metabolic target of environmental contaminants and support the use of RTpiMI and RTdiMI as complementary *in vitro* models to unravel segment-specific toxicodynamic mechanisms in the teleost gut under controlled conditions.

Acknowledgements: This study was supported by Xunta de Galicia.

P-016 - Development of a comprehensive proteome database of sperm and seminal extracellular vesicles in a marine fish: a resource for comparative endocrinology and ecotoxicology research

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Understanding the sperm proteome provides key insights into the proteins underlying the mechanisms involved in fertilisation, and how they differ across individuals of the same species and other species. The gilthead seabream (*Sparus aurata*) is becoming a model marine teleost for studies in comparative reproductive biology and ecotoxicology. However, the available proteomic profile of the seabream sperm is currently very limited. Here, the pooled proteome of different organelles of immature and mature seabream spermatozoa was obtained through shotgun tandem liquid chromatography- mass spectrometry (LC-MS/MS), and analyzed through Gene Ontology (GO). We confidently identified 4650 proteins, from which a total of 4587 GeneIDs were generated leading to 3590 GO terms. When numerically ranked on the basis of annotated proteins, GO analysis indicated that most proteins map to the cytoplasm (31%), membranes (23%), nucleus (18%) and mitochondria (16%). Notable enrichment was also identified in the GO biological process categories of amino acid activation, electron transport chain and translation, and in the GO molecular function categories of electron transfer activity, oxidoreductase activity and structural constituents of ribosomes. Further KEGG pathway analysis returned dominant terms of citrate cycle, aminoacyl-tRNAs biosynthesis, biosynthesis of amino acids and oxidative phosphorylation. Investigation into the extent of conservation between the seabream sperm proteome and those of zebrafish and human identified the presence of 37% orthologous proteins in the three compiled proteomes, while 30% of the proteins were only found in seabream spermatozoa. Among the latter, we found proteins potentially involved in polyubiquitination, cellular respiration and the formation of the cytoskeleton. Current efforts are being directed to identify the massive and dynamic remodeling of the sperm phosphoproteome that drives the hyperosmotic activation of motility of the seabream spermatozoon. In addition, the proteomic composition of the seminal plasma extracellular vesicles, as potential contributors to the post-testicular maturation and storage of spermatozoa, is also being determined by shotgun LC-MS/MS. Altogether, these data will provide a basic resource for further protein-level analysis of seabream sperm enabling an expansion of this area of study in a commonly used model marine organism for reproductive and ecotoxicological research.

Acknowledgements: Supported by the Spanish MICIU (Grant no. PID2022-138066OB-I00, to J.C.), the Catalan AGAUR (Grant 2021 SGR 00068, to J.C.), and the 'Severo Ochoa Centre of Excellence' accreditation (CEX2024-001494-S) funded by the Spanish AEI.

P-017 - Molecular basis of the action of corticosteroids on oogenesis in the teleost fish medaka

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Corticosteroids are crucial in regulating various physiological processes within the body, including stress resistance, osmotic balance, and blood pressure regulation. Consequently, a deficiency of these hormones, primarily resulting from the absence of CYP21A2, a key enzyme in the corticosteroid biosynthesis, is associated with Congenital Adrenal Hyperplasia (CAH). This is a life-threatening condition that, aside from causing severe systemic dysfunctions, also results in reproductive impairments, which have not been extensively studied.

Here, we describe a corticosteroid-deficient medaka resulting from CRISPR/Cas9-induced genetic disruption of the only *cyp21a2* locus (9-base insertion), which recapitulates several systemic defects reported in humans. Although homozygous mutant males did not show any reproductive defects, homozygous mutant females were infertile and never spawned eggs. Posterior phenotypic characterization revealed that although mutant follicles can reach the post-vitellogenic stage, these are retained in the ovary and fail to be ovulated. This was also confirmed by *in vitro* culture and gene expression analyses. Strikingly, spontaneous embryonic activation without fertilization was also observed in the *cyp21a2* homozygous mutant ovary. Since the observed reproductive defects were neither rescued by a preliminary attempt to administer synthetic corticosteroid to late vitellogenic and/or post-vitellogenic follicles in an *in vitro* culture system, nor recapitulated by administration of androgens and 17α-hydroxyprogesterone (a cortisol precursor) to the medaka fish via an abdominal implant and the rearing water, respectively; it is therefore possible that corticosteroids in the medaka fish may play roles during the early/middle stages of oogenesis.

To further investigate the molecular mechanism by which corticosteroids act on medaka oocytes, RNA-seq followed by gene expression screening (RT-qPCR and in situ hybridization) was performed, and several genes were selected for mutagenesis using the CRISPR/Cas9 technique. At this conference, we aim to discuss the mechanisms underlying the phenotypes based on metabolic signatures of steroids, *in vitro* experiments, hormonal treatments, and RNA-seq analyses.

P-018 - Endocrine roles of leptin in gonadotropin regulation and puberty onset in chub mackerel

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Leptin is a key endocrine factor linking nutritional status and reproduction in vertebrates and is essential for puberty onset in mammals. However, despite increasing evidence for the physiological importance of leptin in teleosts, its direct role in the reproductive axis remains poorly understood. In chub mackerel (*Scomber japonicus*), we established a series of experimental systems to investigate the reproductive functions of leptin and its relationship with nutritional status, including recombinant leptin production, primary pituitary cell culture, intracerebroventricular (ICV) administration, long-term *in vivo* treatment, double-labeled in situ hybridization, and a specific sandwich ELISA for circulating leptin measurement.Using primary pituitary cells collected from immature fish, recombinant leptin significantly stimulated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion in a dose-dependent manner. Double-labeled in situ hybridization demonstrated leptin receptor (lepr) expression in both FSH- and LH-producing pituitary cells, indicating that leptin directly regulates gonadotropic cells in teleost fish.

Long-term intramuscular administration of leptin to pre-pubertal females significantly increased gonadosomatic index and oocyte diameter, and approximately 40% of treated fish exhibited vitellogenic oocytes, indicating accelerated pubertal onset. Furthermore, ICV administration significantly increased gnrh1 expression in the preoptic area and fshb and lhb expression in the pituitary, suggesting that leptin activates the brain–pituitary–gonad axis at multiple levels.

To further investigate leptin as a nutritional signal, we established a highly sensitive and specific sandwich ELISA for circulating leptin measurement in chub mackerel. Using this assay system, circulating leptin concentrations were shown to transiently increase after feeding and were significantly higher in sexually mature fish than in immature individuals. These findings support the hypothesis that leptin functions as a metabolic signal informing the reproductive axis that sufficient body energy reserves required for maturation have been attained.

Collectively, our studies provide comprehensive evidence that leptin directly regulates gonadotropin secretion, activates the reproductive axis, and integrates nutritional status with pubertal development and reproductive function in teleost fish.

P-019 - New regulator of reproduction? Secretoneurin localization uncovers strong association with reproductive hormones in brain and pituitary of *Silurana tropicalis*

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Recent research on secretogranin-2 (Scg2) and the proteolytically derived neuropeptide secretoneurin (SN) reveal a critical role in zebrafish reproduction. Although initially discovered in frog brain by Vaudry and Conlon (1991), the potential reproductive functions in amphibians remain unexplored. Immunohistochemistry (IHC) was used to investigate this and the association with classical reproductive peptides by localization of SN within frog brain and pituitary. We generated a new guinea pig polyclonal primary antibody against the *Silurana tropicalis* SN sequence. Sagittal paraffin sections of adult *S. tropicalis* female skulls underwent optimized IHC protocols. Co-staining with antibodies of known reproduction regulators including gonadotropin releasing hormone-1 (Gnrh1), adrenocorticotropic hormone (Acth)/alpha-melanocyte stimulating hormone (a-MSH), arginine vasopressin (Avp), thyroid stimulating hormone (Tsh), luteinizing hormone (Lh), and growth hormone (Gh) was also completed. Specificity of all antibodies was tested and confirmed using pre-adsorption with corresponding antigens/peptides. SN immunoreactivity (ir) appeared within median eminence nerve terminals and throughout the anterior pituitary. Within the pituitary, SN-ir was localized within intermediate lobe melanotrophs and exhibited co-localization in thyrotrophs of the distal lobe. SN-ir was not present in gonadotrophs, but did surround many. SN- ir co-localized to a subset of Avp- and Gnrh1-ir nerve terminals in the inner median eminence, suggesting an association with neurons that produce and release these hormones into portal blood to regulate pituitary function. SN-ir within the median eminence was strongest in the outer layer, indicating it is being secreted to the pituitary gland where it likely interacts with other pituitary hormones. The close association of SN with neuropeptides and pituitary hormones places it as a likely candidate for reproduction regulation and lays the foundation for bioactivity studies in frogs.

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P-020 - Development of a specific AMH ELISA for minimally invasive sex determination in loggerhead sea turtle (*Caretta caretta*)

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Introduction: The loggerhead sea turtle (*Caretta caretta*) is a vulnerable marine species characterized by temperature-dependent sex determination (TSD), in which incubation temperature governs gonadal differentiation. Ongoing global warming is increasingly skewing hatchling sex ratios toward females, posing a significant threat to long-term population viability. In this context, the development of reliable, minimally invasive methods for sex determination is a critical priority for conservation and population management. Anti-Müllerian hormone (AMH), a member of the transforming growth factor-β (TGF-β) superfamily, plays a central role in testicular differentiation and exhibits strongly sexually dimorphic expression, with higher levels in males. A previous study detected AMH in circulation in male C. caretta, supporting its potential as a non-invasive endocrine biomarker for sex identification. The aim of this study was to develop and validate a species-specific enzyme- linked immunosorbent assay (ELISA) for the quantification of circulating AMH in C. caretta.

Material and methods: The amh gene of *C. caretta* was cloned and characterized. Recombinant AMH (ccAMH) was produced in Pichia pastoris and used for the generation of species-specific polyclonal antibodies. Antibody specificity was validated by western blot using both recombinant AMH and protein extracts from testis. An enzyme-linked immunosorbent assay (ELISA) was developed using the P.pastoris derived recombinant AMH and the generated antibodies. To improve assay validation and ensure the use of a structurally relevant antigen, recombinant ccAMH was additionally produced in Chinese hamster ovary (CHO) cells. The optimized ELISA was evaluated using recombinant proteins, testis extracts, and plasma samples from *C. caretta*.

Results and Discussion: The ccamh cDNA was cloned from testicular tissue and sequenced, yielding a 2,034 bp sequence consistent with predictions. Western blot analysis of testis extracts revealed a protein of approximately 55 kDa, confirming the predicted size of the ccAMH. Comparative analysis highlighted notable divergence among vertebrate AMHs, with reptiles characterized by longer signal peptides and extended N-terminal regions, while the C-terminal TGF-β domain remains highly conserved, including key cysteine residues. Phylogenetic analysis grouped amh genes from reptiles together with birds, distinct from mammals, confirming a closer evolutionary relationship between these lineages. Based on the gene sequence, protein characteristics, and phylogenetic insights, a recombinant ccAMH construct was designed for heterologous expression.

Recombinant AMH was successfully produced in P. pastoris and enabled the generation of polyclonal antibodies with high specificity toward C. caretta AMH. Western blot analysis confirmed antibody recognition of both recombinant protein and endogenous AMH in testis extracts, with bands corresponding to the expected molecular weight. Key assay parameters, including antigen coating concentration, antibody dilution, incubation conditions, and blocking strategies, were systematically optimized. Assay sensitivity, specificity, and reproducibility were assessed to confirm its suitability for detecting circulating AMH.

Conclusion: Overall, this study establishes the first species-specific ELISA for AMH in *C. caretta*, providing a minimally invasive and practical tool for sex identification. This approach represents a significant advance in the application of comparative endocrinology to wildlife conservation and offers valuable opportunities for monitoring population dynamics and assessing the impacts of climate change on TSD species.

Funding: This research was part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana through GVA-THINKINAZUL/2021/42 (PI: A. Gómez) and the Prometeo grant CIPROM/2024/2 (CLIMEDREP).

P-021 - Spatial transcriptomics profiling in European sea bass (*Dicentrarchus labrax*) in maturing testis

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Controlling reproduction remains a major challenge in fish aquaculture, particularly in European sea bass (*Dicentrarchus labrax*), where males frequently reach puberty precociously. Early maturation diverts energy towards reproductive processes at the expense of somatic growth, negatively affecting flesh quality and generating significant economic losses for the industry. The onset of male puberty depends on tightly regulated interactions between germ cells and their surrounding somatic environment. However, conventional omic approaches usually rely on bulk tissue analysis, masking cellular heterogeneity and limiting spatially restricted molecular processes. Spatial transcriptomics overcomes these limitations by enabling the characterization of gene expression directly within tissue sections while preserving spatial organisation.

In this study, we reanalysed previously generated spatial transcriptomic datasets from four European sea bass testes at early maturation stages (Stage I to Stage II–III). Tissue sections were processed using 10x Genomics Visium slides, sequenced on a NovaSeq platform, and raw sequencing data preprocessed with 10x Genomics Space Ranger v2.0.0. To explore spatial gene expression patterns, we implemented a reproducible bioinformatics workflow integrating complementary analytical approaches to increase robustness and reproducibility. Exploratory analyses performed with the 10x Genomics Loupe Browser (v9.0.0) were compared with a customizable pipeline implemented in Seurat v5 in R. Within Seurat, low quality spots and features were filtered out, and normalisation was performed using SCTransform to correct for technical variability (i.e. sequencing depth) while preserving biological signals. Dimensionality reduction was conducted using principal component analysis (PCA) followed by the construction of a shared nearest-neighbor (SNN) graph and unsupervised clustering using the Louvain algorithm.

Preliminary results revealed a clear spatial segregation between germinal regions and epithelial or vascular compartments within the testis. In addition, spatial autocorrelation analyses using Moran's I suggest that gene expression patterns are not randomly distributed across the tissue, but instead appeared associated with specific regions. Ongoing analysis focusses on refining analytical pipelines and improving the identification and validation of differentially expressed genes across spatial regions. Future work will aim to characterise key signaling pathways and local cellular environments involved in triggering meiosis during puberty. Overall, these findings highlight the power of spatial transcriptomics to resolve tissue-specific regulatory mechanisms and identify novelcandidate molecular markers to improve reproductive control strategies in aquaculture.

Funding: Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033, Grants PID2021-122929OB-C31, PID2021-122929OB-C33, CEX2018-000794-S

P-022 - Involvement of the transcriptional coactivator Ncoa7 in early gametogenesis and puberty of European sea bass (*Dicentrarchus labrax)*

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Introduction: Puberty and gametogenesis are complex developmental processes regulated by endocrine and local molecular mechanisms that remain poorly understood in teleost fish. Previous studies in European sea bass (*Dicentrarchus labrax*) using hemigonadectomy and transcriptomic approaches identified ncoa7 as a candidate gene involved in the early events of gonadal maturation. Ncoa7 encodes a nuclear receptor coactivator that, in mammals, enhances the transcriptional activity of steroid hormone receptors, particularly estrogen receptors. However, its role in fish reproduction remains largely unknown. This study aimed to characterize the molecular and functional role of Ncoa7 during early gametogenesis and puberty in European sea bass and to determine its interaction with estrogen, androgen and progesterone signaling pathways in both sexes.

Materials and Methods: The complete cDNA sequence of sea bass ncoa7 was cloned and functionally characterized. Tissue distribution and expression patterns across the reproductive cycle were analyzed by RT-qPCR in male and female gonads , together with steroid receptor genes (*esr1*, *esr2a* and *esr2b*). Functional studies were performed in HEK293 cells co-transfected with sea bass Ncoa7 and estrogen, androgen or progesterone receptors, followed by stimulation with their respective ligands.

To investigate the role of Ncoa7 during puberty, a hemigonadectomy approach was used in female sea bass to identify early molecular events preceding precocious maturation. Hormonal profiles and gene expression patterns were compared between precocious and non-precocious females. In males, ncoa7 expression was analyzed throughout the reproductive cycle, and in *in vitro* cultures of immature testes treated with estradiol (E2) or recombinant Fsh. Localization of Ncoa7 and Esr1 was examined by immunofluorescence.

Results and Discussion: ncoa7 was highly expressed in brain and gonadal tissues of both sexes and significantly enhanced the transcriptional activity of estrogen, androgen and progesterone receptors, demonstrating its role as a multifunctional steroid receptor coactivator.

In females, hemigonadectomy experiments revealed distinct endocrine and transcriptomic profiles between precocious and non-precocious fish. Increased ncoa7 expression during vitellogenesis and early ovarian development coincided with elevated steroid hormone signaling, suggesting a role for Ncoa7 in the initiation of puberty. Functional data support the hypothesis that Ncoa7 may interact with progesterone and estrogen pathways to regulate the onset of ovarian maturation.

In males, ncoa7 expression peaked in immature testes and overlapped with high esr1 expression and elevated plasma E2 levels. Ncoa7 and Esr1 were co-localized in type A spermatogonia. Moreover, E2 stimulated type A spermatogonial proliferation, whereas recombinant Fsh downregulated both ncoa7 and esr1 expression. These findings indicate that the Ncoa7–Esr1 complex participates in the regulation of early spermatogonial proliferation and may contribute to controlling the onset of spermatogenesis. Overall, the results identify Ncoa7 as a key regulator of steroid receptor signaling during early gametogenesis in European sea bass. Its involvement in both ovarian puberty and

spermatogonial proliferation suggests a central role in coordinating gonadal development and reproductive maturation in this species.

Funding: MCIN/AEI/10.13039/501100011033/ and ERDF a way of making Europe (RTI2018-094667-B-C22; PID2025-168600OB-C31).

P-023 - Modulation of juvenile rainbow trout endocrine stress response under cumulative stressors: implications for fish welfare

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The endocrine stress response is activated in the presence of a stressor. Its intensity, duration and frequency determine how it develops, as it constitutes an adaptive mechanism whose reorganisation depends on previous experiences and the general condition of the individual. In fish reared in captivity, whether for scientific or productive purposes, individuals face stressors of varying nature and intensity that act in a complementary and cumulative manner. However, evidence regarding how the endocrine response is triggered under simultaneous or cumulative exposure to multiple stressors is still limited. This study evaluated how juvenile rainbow trout respond to different stressor, considering their intensity, duration and temporal repetition, and how these conditions affect the activation of the endocrine stress axis. Over three months, groups of fish were subjected to four treatments: 1) Control group: minimal handling operations. 2) High-density group: progressive monthly increase in stocking density (20, 30 and 40 kg/m^3). 3) Handling stress group: aerial exposure for 30 seconds, twice weekly. 4) Combined group: simultaneous exposure to hypoxia and progressive density increase. Biometric and haematological parameters were analysed at four sampling points: time 0 (baseline), and 1, 2 and 3 months after the start of the trial. The results show that growth was affected in the medium term, being significantly lower in fish exposed to combined stressors. The hepatosomatic index decreased in fish subjected to high density, suggesting increased metabolic mobilisation, whereas the splenic index was more affected by handling stress. Regarding haematological parameters, handling stress had a stronger effect on plasma glucose and ions, while density primarily influenced haemoglobin and haematocrit. The combination of both stressors produced cumulative effects on most parameters, notably causing a marked increase in blood HCO_3^-. Our results suggest that repeated exposure to different stressors in juvenile rainbow trout generates specific and cumulative physiological responses. Handling operations and high stocking density differently affect growth, somatic indices and haematological parameters, while their combination amplifies the activation of the stress response, emphasising the need for careful management in captivity. This study was funded by MARINNONET project (European Union, INTERREG Atlantic Area 2021-2027, Ref. MARINNONET-EAPA_0017/2022)

P-024 - Sexual dimorphism in the epigenetic and transcriptional regulation of gonadal stress responses in fish

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Environmental and biological stressors are major challenges in aquaculture, affecting animal welfare, reproductive performance, disease susceptibility, and productivity. Increasing evidence indicates that stress responses are regulated through complex interactions between endocrine, immune, and epigenetic pathways, which may differ between males and females. In this study, we investigated sex-specific molecular and cellular regulatory mechanisms in gonadal tissues following an acute immune challenge, using the European sea bass (*Dicentrarchus labrax*) as a teleost model relevant to aquaculture. We focused on the relationship between DNA methylation and gene expression to better understand how stress-related signals are integrated at the molecular level. Methylome, transcriptome and miRNome profiles were analysed using sequencing strategies. Particular attention was given to genes involved in cellular stress responses, immune regulation, tissue remodeling, and signaling pathways associated with gonadal function and endocrine regulation. Several genes associated with cellular signaling, stress adaptation, immune function, and tissue homeostasis exhibited sexually dimorphic methylation patterns. Some candidates displayed opposite methylation profiles between ovaries and testes, whereas others showed coordinated changes in methylation and expression exclusively in one gonadal tissue. Notably, sexual dimorphism was already evident under basal conditions, with ovaries generally showing lower methylation levels in selected genes together with a tendency towards lower expression. Following the challenge, ovaries exhibited stable or increased expression of these genes, whereas testes showed a general downregulation trend. These findings demonstrate that acute stress-related stimuli trigger sex-specific epigenetic and transcriptional responses in the gonads, highlighting the existence of distinct molecular and cellular regulatory mechanisms between males and females. The results support a key role for DNA methylation in mediating endocrine–immune crosstalk and stress adaptation and provide new insights into the molecular basis of sexual dimorphism in vertebrate stress responses. Understanding these regulatory processes may contribute to the development of biomarkers of resilience and welfare, supporting more sustainable and efficient aquaculture production systems.

P-025 - Endocrine regulation of stress axis in the teleost fish gilthead seabream (*Sparus aurata*) exposed to high stocking density and dietary nanoplastics

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Aquaculture intensification exposes cultured fish to multiple stressors that may compromise their physiological performance and welfare. Among the most relevant are high stocking density and the increasing presence of emerging contaminants such as nanoplastics (NPs) in the aquatic environment and aquafeed. In teleost, stress responses are mainly mediated through activation of the hypothalamic–pituitary–interrenal (HPI) axis, leading to cortisol release. Sustained cortisol elevation can alter neuroendocrine homeostasis and modulate corticosteroid signaling pathways, affecting the capacity of fish to properly cope with prolonged stress exposure. Although both stocking density and NP exposure have individually been associated with endocrine disruption, little is known about their combined effects on the molecular regulation of stress- related pathways in marine aquaculture species.

The present study aims to evaluate the combined effects of stocking density and dietary NP exposure on the molecular regulation of the HPI axis in juvenile gilthead seabream (*Sparus aurata*), with the objective of identifying sensitive biomarkers associated with stress adaptation and fish welfare under conditions of high stocking density and NP exposure.

Juvenile fish with an initial body weight of approximately 100 g were randomly distributed into 12 experimental tanks under open-flow conditions following a 2 × 2 factorial design. Fish were exposed for 64 days to four experimental conditions in triplicate: (i) low stocking density (5 kg m^{-3}) with control diet (LSD-CT), (ii) low stocking density with NP-supplemented diet (LSD-NPs), (iii) high stocking density (16 kg m^{-3}) with control diet (HSD-CT), and (iv) high stocking density with NP- supplemented diet (HSD-NPs). The NP diet contained spherical polystyrene nanoparticles (50 nm diameter; Bang Laboratories) at a concentration of 50 mg kg^{-1} feed. Environmental conditions were maintained constant throughout the trial (21–22 °C; natural photoperiod (September–December) in southern Spain).

At the end of the exposure period, fish were sampled to obtain plasma and several stress biomarkers (cortisol, glucose and lactate) were determined. In addition, tissues involved in neuroendocrine regulation were obtained, including brain, pituitary, and head kidney. Total RNA was extracted and reverse transcribed for quantitative real-time PCR (qPCR) analyses. Gene expression of HPI axis components was analyzed in a tissue-specific manner. In the brain, thyrotropin-releasing hormone (*trh*), corticotropin-releasing hormone (*crh*) and its binding protein (*crhbp*) were evaluated. In the pituitary, proopiomelanocortin isoforms (*pomca*, *pomcb*, and *pomcl*) were analyzed. In the head kidney, steroidogenesis- and cortisol metabolism-related genes were assessed, including *star*, *cyp11c1*, and *hsd11b2*. Corticosteroid receptor expression was also measured, including glucocorticoid and mineralocorticoid receptors (*nr3c1* and *nr3c2*).

We hypothesize that the combined exposure to HSD and dietary NPs will induce an enhanced stress response characterized by the upregulation of key HPI-related genes, particularly in the HSD-NPs group. Additionally, sustained stress conditions may alter corticosteroid receptor expression,

suggesting adaptive or desensitization mechanisms at the endocrine level that could reflect changes in stress sensitivity.

This study is expected to provide an integrative understanding of the molecular mechanisms involved in multifactorial stress adaptation in S. aurata. Furthermore, the identification of endocrine and molecular biomarkers may contribute to the development of more sensitive indicators of fish welfare and resilience in Mediterranean aquaculture systems exposed to intensive production practices and emerging contaminants.

Acknowledgements: This work was supported by the project PID2023-149326OB-C22, funded by MICIU/AEI (10.13039/501100011033) and co-funded by European Union through the ERDF (European Regional Development Fund).

P-026 - Modulation of stocking density stress response in European seabass (*Dicentrarchus labrax*) through dietary phytogenic supplementation

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Inadequate stocking densities often compromise the welfare and growth of fish. Functional feed additives with stress- modulating properties are gaining attention as a tool to mitigate the physiological impact of intensive farming. This study evaluates the efficacy of a phytogenic additive with relaxing properties (RELAQUAX, Bedson España S.L.) in the diet of European seabass (*Dicentrarchus labrax*) reared under different stocking density conditions. Juvenile fish (n = 713; 30 g initial weight) were reared for a period of 87 days in 500-L tanks under four replicate conditions: two diets (control and supplemented with RELAQUAX at 0.2 %) and two initial stocking densities adjusted by the number of fish and the effective volume of the tank (low stocking density, LSD, 6 kg·m-3; and high stocking density, HSD, 30 kg·m-3). Following satiation feeding under controlled temperature and photoperiod, biometric data were recorded periodically (at 21, 42, 59 and 87 days) to determine growth parameters using PIT-tags. Finally, samples (brain, pituitary, head kidney and plasma) were collected to analyse stress indicators. A two-factor ANOVA was performed, following the removal of outliers (Tukey´s IQR method) and verification of assumptions. Where significant interactions were present ($p < 0.05$), comparisons of simple effects were carried out using Holm's post-hoc test.

Including RELAQUAX in the aquafeed did not negatively impact fish growth under LSD condition. The results showed significant interactions between diet and density at 42 and 59 days. HSD reduced weight gain (WG) regardless of diet. However, RELAQUAX significantly improved WG under HSD condition respect to control diet. Plasma cortisol levels did not increase under HSD and control diet; however, it enhanced significantly in animals supplemented with RELAQUAX regardless of stocking density. These results suggest that hypothalamic-pituitary-interrenal (HPI) axis is saturated in control group, whereas supplementation with RELAQUAX has enabled an optimal response to density-induced stress to be maintained, as evidenced by the higher WG under HSD conditions respect to control group during the periods indicated above.

In the brain, thyrotropin-releasing hormone (*trh*), corticotropin-releasing hormone (*crh*) and its binding protein (*crhbp*) were evaluated to map central stress signalling. In the pituitary, proopiomelanocortin (*pomc*) was analysed as the primary precursor for ACTH. In the head kidney, steroidogenesis- and cortisol metabolism-related genes were assessed, including steroidogenic acute regulatory protein (*star*), which controls the rate-limiting step of cholesterol transport, and P450 11β-hydroxylase (*cyp11b1*), the enzyme responsible for final cortisol synthesis. Corticosteroid receptor expression was also measured, including glucocorticoid and mineralocorticoid receptors (*nr3c1* and *nr3c2*), to determine peripheral tissue sensitivity to these hormonal signals under the experimental conditions here tested. The results will be analysed to determine the influence of HSD condition on HPI axis response, as well as to understand how dietary phytogenic supplementation with the additive RELAQUAX could modulate the activation of this axis due to HSD condition. This study is expected to provide a comprehensive understanding of the molecular mechanisms involved in adaptation to HSD stress and its modulation by the RELAQUAX additive in D. labrax.

Acknowledgements: Project PHYTOWELFISH (PCM_00029) co-funded by “Consejería de Universidad, Investigación e Innovación” from “Junta de Andalucía” and by European Union through Next Generation EU funds of the “Plan de Recuperación, Transformación y Resiliencia” from Spanish Government. A. Blázquez-Durán has an FPU grant from Spanish Ministry of Universities (FPU23/03155). The company Bedson España S.L. (Spain) provided the feed additive RELAQUAX.

P-027 - Coordinated thyroid axis dynamics describes metamorphosis in the flathead grey mullet (*Mugil cephalus*)

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The flathead grey mullet (*Mugil cephalus*) is a widely distributed euryhaline teleost of ecological and economic importance whose prolonged larval phase and metamorphosis are associated with high mortality and variable growth, limiting commercial production. To elucidate the regulation of metamorphic development, we characterized the ontogeny of the hypothalamic–pituitary–thyroid (HPT) axis, the central regulator of teleost metamorphosis. Thyroid follicles began producing T3 and T4 at first feeding, with follicle number and area increasing markedly from metamorphosis onset. Whole-body hormone levels showed a T4 peak at metamorphosis initiation and a T3 peak at its climax. Gene expression revealed early activation of hypothalamic (*trh, crhb*) and pituitary (*tshba, galr1a*, *crhr1, crhr2*) components, followed by increased expression of thyroidal genes (*tg, dio2*) and thyroid hormone receptors (*thrb, thrab*) during metamorphosis. Together, these data demonstrate a coordinated endocrine program aligning hormone production, receptor availability, and axis regulation to drive timely metamorphic progression, providing a reference framework for thyroid axis function in *M. cephalus*.

8. Endocrine disruption

P-028 - Hepatic miRNome and endocrine-related molecular responses induced by environmentally exposed microplastics in gilthead seabream (*Sparus aurata*)

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The increasing accumulation of microplastics (MPs) in aquatic ecosystems has raised concern regarding their role as vectors of environmental contaminants and their potential impact on aquatic organisms. Although several studies have investigated the toxicological effects of MPs and associated contaminants, little is known about the involvement of microRNA-mediated regulatory mechanisms in fish exposed to environmentally contaminated MPs under realistic exposure scenarios. In the present study, a mixture of polyethylene (PE-HD and PE-LD), polypropylene (PP) and polystyrene (PS) microplastics was environmentally exposed for 37 days at the mouth of the Chienti River (Central Adriatic Sea, Italy), an area subjected to anthropogenic and industrial inputs. After environmental exposure, MPs showed a marked increase in mass (mean +24%), likely associated with biofilm formation and accumulation of particulate organic matter. Chemical characterization revealed the presence of several classes of contaminants adsorbed onto MPs, including perfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAHs) and pesticides. Among PFAS, PFOA was the predominant compound, although detected at relatively low concentrations (up to 0.253 µg/kg). In contrast, pesticides represented the quantitatively dominant contaminant class, reaching concentrations up to 22.98 µg/g for Mocap and 25.91 µg/g for hexachlorobenzene. Furthermore, PAHs such as benzo(b)fluoranthene reached concentrations up to 11.88 ng/g, particularly on PS particles. To evaluate the biological effects associated with contaminated MPs, juvenile *S. aurata* were exposed for 30 days through dietary administration (1% MPs in feed) under three experimental conditions: control diet, pristine MPs and environmentally exposed MPs. Preliminary molecular docking simulations performed with the Endocrine Disruptome Tool predicted moderate to high binding affinities of several detected contaminants, particularly benzo(b)fluoranthene and PFOA, towards nuclear receptors ligand-binding domains, supporting the potential endocrine-disrupting activity of environmentally exposed MPs. Hepatic transcriptional analyses were performed by quantitative Real-Time PCR targeting genes involved in xenobiotic sensing, endocrine signaling and lipid metabolism. Fish exposed to environmental MPs showed significant up-regulation of PPARα, RXRα, AHR1, PXR and ERα, together with increased expression of vitellogenin (VTG). Conversely, a significant down- regulation of APOA1 and hepatic lipase (HL) was observed. The combined activation of xenobiotic receptors (AHR1 and PXR) and lipid-related nuclear receptors (PPARα/RXRα) supports the hypothesis of a coordinated hepatic response to contaminant mixtures associated with the plastisphere. In parallel, total hepatic bile acids (TBA) were significantly increased in fish exposed to environmental MPs, suggesting an alteration of hepatic lipid and bile acid homeostasis. Additionally, these results were further integrated with hepatic miRNome profiling in order to investigate the possible involvement of microRNA- mediated regulatory mechanisms in the observed molecular responses. Preliminary analyses suggest differential modulation of several hepatic miRNAs associated with xenobiotic metabolism, endocrine signaling and

lipid homeostasis pathways. Overall, our findings highlight the role of environmentally exposed MPs as carriers of complex contaminant mixtures capable of modulating hepatic endocrine and metabolic pathways in marine fish, emphasizing the importance of considering realistic environmental exposures in ecotoxicological studies. Furthermore, our results suggest that miRNA-mediated regulation may represent an important mechanistic component of the molecular response to MPs-associated contaminants in aquatic organisms.

P-029 - Comparative proteomics to investigate neuroendocrine-related molecular responses in *Daphnia magna*

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Aquatic organisms respond to neuroactive chemicals through coordinated molecular changes affecting metabolism, reproduction, development and endocrine-related signalling. Understanding these responses is essential for environmental toxicology and comparative endocrinology, particularly in species widely used as models for aquatic risk assessment. Daphnia magna is a key freshwater invertebrate model to investigate environmental challenges and potential neuro- endocrine disruption, but proteomic studies in this organism can be limited by low sample amount, matrix complexity and workflow-dependent variability. Here, we establish a comparative proteomic approach to investigate endocrine molecular responses in this aquatic model organism.

To support this aim, two LC-MS/MS sample-introduction strategies were evaluated: direct injection and trap-and-elute. Protein extracts were enzymatically digested, purified, reconstituted and analysed by high-resolution Orbitrap mass spectrometry. Workflow performance was assessed in terms of protein identification, peptide coverage, analytical robustness and suitability for complex biological samples.

Direct injection provided the highest proteome coverage, with 3,305 protein groups identified. The trap-and-elute workflow identified 2,753 protein groups, showing a moderate reduction in total identifications compared with direct injection. This trade-off is consistent with trap-based configurations, where some peptides, particularly highly hydrophilic ones, may be lost. However, trap-and-elute offers important advantages for biological proteomics, including sample clean-up before the analytical column, reduced matrix load, improved reproducibility and robustness, and extended analytical column lifetime. These features make it particularly suitable for routine and large-scale studies using complex samples from small aquatic organisms.

Importantly, the optimised workflows enabled the detection of proteins relevant to endocrine and reproductive biology in Daphnia magna, including vitellogenins, juvenile hormone-associated proteins, steroid dehydrogenases, insulin-related proteins and nuclear receptor cofactors. These protein classes support the relevance of the approach for future studies addressing reproduction, development, metabolism and endocrine disruption.

As a proof of concept, the trap-and-elute workflow was further applied to Daphnia magna samples exposed to neurotoxic compounds known to destroy dopaminergic neurons and produce parkinsonism like phenotypes: MPTP and rotenone. This exposure experiment provides an initial application of the optimised workflow to neuro-endocrine physiologically relevant stress conditions, supporting its use for future identification of protein signatures associated with toxicant exposure, stress regulation and endocrine-related molecular responses.

Overall, this work establishes a proteomic platform for studies in Daphnia magna. By combining broad proteome coverage with improved robustness for complex samples, these workflows provide a methodological basis to investigate endocrine and stress-associated molecular responses in environmental and comparative endocrinology. Future research will be to develop an analytical framework to determine neuropeptides.

P-030 - Decoding the molecular fingerprints of organophosphate flame retardants with transcriptomics

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Organophosphate flame retardants (OPFRs) are a diverse group of chemicals used to make materials less flammable. Because of their structural and physicochemical diversity, including differences in polarity, solubility and persistence, they can cause a wide range of toxic effects, such as neurotoxicity, hepatotoxicity, metabolic disorders, and alterations in reproductive pathways, among others.

In this study, we investigated the effects of 10 OPFRs: Triphenyl phosphate (TPhP), tri(2-butoxyethyl) phosphate (TBOEP), Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(1-chloro-2-propyl) phosphate (TCIPP), tri-n-butyl phosphate (TNBP), tetrabromobisphenol A (TBBPA), 2,4,6-tribromophenol (2,4,6-TBP), hexabromobencene (HBB), 2,3,4,5,6-pentabromotoluene (PBT), 2-ethylhexyl diphenyl phosphate (EHDPP) in zebrafish embryos. Exposures were performed from 4 to 5 days post-fertilisation, and effects on developmental parameters and survival rates were evaluated. Samples were collected for a dose- response analysis (0, 8.23, 28.27, 100.6, 351, 1230 µg/L) using a high-throughput microfluidic qPCR platform to quantify 96 genes previously selected. Also, several locomotor activities were analysed using a behaviour testing chamber (DanioVision, Noldus Information Technology, Leesburg, VA), including startle response, non-associative habituation, basal locomotor activity, and visuomotor response. To simulate the presence of danger, vibratory and visual stimuli were applied.

Behavioural responses in zebrafish larvae revealed endpoint- and compound-specific neurotoxicity, with the startle response emerging as the most sensitive endpoint showed distinct behavioural toxicity profiles, highlighting variability within OPFRs. TPHP was the most disruptive, primarily targeting mechanosensory and motor circuits (startle, habituation, locomotion). Our results also showed that the startle response was the most sensitive indicator of sublethal neurodevelopmental toxicity in aquatic organisms. Analysis of transcriptomic responses is currently ongoing.

Overall, our findings highlight that OPFRs act through compound-specific mechanisms and that their molecular diversity plays a key role in their toxicity.

Funding: Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033, Grants: CEX2018-000794-S, CNS2022-135257

P-031 - Divergent modes of action converge on shared adverse outcomes: a multi-level analysis of BPA, PFOS, and TBT

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Endocrine-disrupting chemicals (EDCs) can perturb biological systems at molecular levels before phenotypic effects are observed, complicating their integration into hazard assessment. Here, we applied a multi-level framework to characterize and compare the toxicological profiles of bisphenol A (BPA), perfluorooctane sulfonate (PFOS), and tributyltin (TBT) in zebrafish embryos. Literature based-transcriptomic, metabolomic, and morphometric data were analyzed using benchmark concentration modeling to derive points of departure (PoDs) across biological levels. Transcriptomic PoDs occurred at concentrations up to two orders of magnitude lower than morphological effects. Absolute transcriptomic PoDs revealed a molecular potency ranking of TBT (0.0093 µM) > PFOS (0.021 µM) > BPA (0.135 µM), while equilethal normalization demonstrated that BPA and PFOS induced extensive sublethal molecular perturbations at substantially lower fractions of their lethal concentrations when compared to TBT. At the pathway level, each compound exhibited distinct but partially overlapping biological signatures. BPA exposure primarily affected lipid metabolism and transport, retinoid signaling, and visual perception pathways, indicating early disruption of developmental and metabolic regulation. PFOS responses were dominated by alterations in cell-cell communication, immune and cytokine signaling, apoptosis, and lipid utilization processes, consistent with shifts in energy expenditure. In contrast, TBT elicited strong perturbations in cell cycle regulation, DNA replication, chromatin organization, and steroid biosynthesis, reflecting widespread disruption of fundamental cellular functions. Despite these differences, pairwise and integrative analyses revealed a core set of shared responses across compounds, including lipid metabolism, oxidative stress, energy homeostasis, and autophagy-related pathways. A conserved group of 45 differentially expressed genes, involved in processes such as lipid regulation (e.g., *angptl3*, *soat2*), stress response (e.g., *prdx1*), and cell cycle control (e.g., *e2f1*), highlights common molecular signatures of exposure. These shared and compound-specific responses provide a basis for identifying both general and chemical-specific biomarkers of endocrine disruption. Integration of molecular responses with downstream phenotypes enabled the development of adverse outcome pathway (AOP) networks describing distinct mechanistic trajectories for each compound. BPA acts through multi-receptor interactions affecting lipid and retinoid regulation, PFOS primarily modulates lipid catabolism and immune signaling, whereas TBT disrupts cell cycle progression and steroid metabolism through retinoid X receptor-mediated pathways. Despite divergent molecular initiating events, all three EDCs converged on key regulatory axes related to lipid metabolism, energy homeostasis, and developmental regulation. Overall, this study demonstrates how multi-level integration combined with PoD analysis can support AOP network development and improve mechanism-based hazard characterization within new approach methodologies (NAMs).

P-032 - Revisiting the role of prolactin in amphibian metamorphosis

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Amphibian metamorphosis is a critical postembryonic process during which aquatic larvae undergo profound physiological, morphological and ecological transformations to become terrestrial or semi-terrestrial frogs. This transition is regulated primarily by thyroid hormones (THs), which orchestrate tissue-specific reprogramming processes by inducing cellular proliferation, migration, and apoptosis. More recently, corticosteroids (CS) have been identified as key modulators of TH action, highlighting the importance of hormonal crosstalk during metamorphosis. Prolactin (PRL), one of the most versatile hormones among vertebrates, has also emerged as a relevant hormonal regulator of this developmental transition. Early experimental evidence showed that PRL can inhibit tail resorption, a crucial process for metamorphic progression, suggesting an antagonistic role against THs. This hypothesis has since regained attention following toxicological research demonstrating that disruption of PRL signaling can alter metamorphic progression, further underscoring the complexity of hormonal crosstalk occurring during this process. Moreover, an additional *prl* paralog has recently been identified in amphibians, derived from a gene duplication linked to major osmotic transitions in early tetrapods. The retention of both gene copies further supports the evolutionary and physiological significance of PRL in this group.

The objective of this research project is to better characterize PRL actions and its interactions with THs and CS during amphibian metamorphosis, while addressing major knowledge gaps regarding PRL expression, regulation, and function using modern molecular approaches. To address this, the expression of both *prl* genes, as well as genes involved in PRL regulation, was measured in the brain, tail, gills, lungs, and intestine of *Xenopus* (*Silurana*) *tropicalis* tadpoles at Nieuwkoop- Faber developmental stages 54, 58, 62, 64, and 66. Prolactin receptor (*prlr*) was also characterized across tissues to better asses tissue-specific responsiveness to PRL signaling. Developmental expression profiles of both *prl* genes revealed potential divergent regulation between paralogs and tissue-specific expression patterns, including in the brain, which contains the pituitary, the principal known source of PRL. Importantly, the characterization of the second *prl* paralog in extrapituitary tissues supports the hypothesis of functional specialization between paralogs. Moreover, tissue-specific *prlr* expression patterns suggest differential PRL requirement among tissues, potentially reflecting distinct physiological roles of PRL during metamorphosis.

Together, these findings highlight the need to further investigate paralog-specific interactions with THs and CS to advance our understanding of PRL signaling during amphibian metamorphosis.

P-033 - Role of metalloproteinases in folliculogenesis in zebrafish

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ADAMTS9 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 9) is a conserved and broadly expressed extracellular matrix metalloprotease that is critical for the development of various organs including ovaries. Loss of Adamts9 causes male-biased sex development, underdeveloped ovaries, female infertility, and intersex phenotype. Adamts9 is expressed in ovarian stroma, primary follicles, stage I follicles, and preovulatory follicles in juveniles and adults in zebrafish. In contrast to continued development through later stages of follicles (Stage II, III) in wildtype, whereas most follicles in Adamts9 KO sibling remain arrested as primary follicles and do not progress further in mid- or late-juveniles. Additionally, we also found morphological evidence of active sex reversal in Adamts9 KO including degenerating follicles or coexistence of ovarian follicles and spermatic cyst with mature sperm in the same gonad. By knocking out tp53, a major apoptosis pathway, in Adamts9 KO, we were able to partially rescue the underdeveloped ovaries. Finally, RNAseq analysis found significant disruption of chemokine and cytokine signaling in KO adult ovary, dysregulated ECM during early oogenesis. Taken together, we demonstrated that adamts9 expression is critical for the development of primary ovarian follicles and sex determination in juvenile zebrafish.

P-034 - Spontaneous precocious vitellogenesis of female *Anguilla rostrata* in captive seawater cage

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Northern hemisphere temperate Anguillid eels, including *Anguilla rostrata*, *A. anguilla* and *A. japonica*, typically exhibit ovarian development arrested at the oil droplet stage prior to seaward migration, with final maturation occurring during oceanic spawning migration. Although tropical anguillid eels can reach early to mid-vitellogenesis before the onset of spawning migration, spontaneous early vitellogenesis of captive-reared populations has not been documented. Here, we report large-scale spontaneous vitellogenesis in A. rostrata reared in a nearshore sea cage. Over 40% of the 2-year-old *A. rostrata* developed to the early vitellogenic (EV) stage with a mean follicle diameter of 353.9 ± 42.3 μm and a gonadosomatic index exceeding 4%. In these fish, plasma levels of estradiol and 11-ketotestosterone, as well as key genes in the brain-pituitary-gonad-liver (BPGL) reproductive axis—including *gnrh1*, *fshb*, *lhb*, *lhr*, *cyp19a1*, and *vg1/2*—were significantly higher than those in fish arrested at the early oil droplet (EOD) stage. Transcriptomic analysis comparing brains from eels at the EOD and EV stage identified 14 significantly upregulated genes and 12 significantly downregulated genes. Among these, the significant upregulation of neuroendocrine factors such as *galp*, *cyp19a1*, and *dexras1* was confirmed by qPCR. These neuroendocrine factors likely integrate physiological and environmental cues to activate the BPGL axis and facilitate precocious vitellogenesis in *A. rostrata*. Furthermore, eels that had undergone spontaneous vitellogenesis required only 6–7 weekly hormone injections to be successfully induced to maturation, suggesting that they were more suitable for artificial reproduction. Overall, our findings advance the understanding of reproductive initiation in A. rostrata and support strategies to improve ovarian development under aquaculture conditions.

P-035 - Bioluminescence of the large yellow croaker: involvement of xanthophore's fluorescence in physiological luminescent color tuning

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Bioluminescence evolved frequently in marine fish. They are abundant in deep-sea, rather than shallow water. The large yellow croaker, *Larimichthys crocea* (Teleostei: Sciaenidae), inhabits coastal waters at 30-100 meters. This fish is known as a prominent aquaculture species in China, valued for its golden yellow coloration. Despite the extensive aquacultural studies, its bioluminescence properties remain totally undocumented. In this study, we discovered that the bioluminescence from patch-like photophores on the ventral body surface can be induced by the intraperitoneal injection of adrenaline. The luminescence was readily visible to the human naked eyes in the dark. The bioluminescent color was blue (lmax, 458 nm) when xanthophore's yellow pigments were aggregated in spherical structures on the photophores, while green (lmax, 458 and 528 nm) when the pigments were dendritically dispersed by the pre-injection of Melanotan Ⅱ, an analog of α-MSH. The dendritic yellow pigment possessed a green fluorescence at the emission max 525 nm, suggesting the involvement of energy transfer of the blue luminescence to the fluorescent pigment to give green luminescence. The bioluminescence can also be induced by melatonin injection or by physically stroking. These results suggested that the biological function of the ventral bioluminescence is primarily counterillumination; the hormonal regulation of the luminescence color will be an adaptation to their habitat environments. Physical contact-induced bioluminescence may be related to their schooling behavior. A similar bioluminescence was also observed in the other sciaenid species, *Larimichthys polyactis* and *Collichthys lucidus*. The present study provided convincing evidence of bioluminescent fish species in Sciaenidae.

P-036 - Modulation of fish carboxylesterases by early-life exposure to emerging contaminants

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While numerous studies have focused on single-compound effects in fish embryos, assessing chemical mixtures is of particular environmental relevance. Concurrently, growing concerns about conventional plastics have accelerated the adoption of biodegradable plastics (BPs). However, BPs can release potentially harmful chemicals during degradation. Carboxylesterases (CEs) are key enzymes involved in the hydrolysis of a wide variety of substrates, including fatty acids, hormones and xenobiotics. Because of their broad substrate specificity, CEs play crucial roles in both detoxification and physiological processes such as lipid metabolism. Therefore, this study aimed to evaluate the effects of two BP extracts and a mixture of plastic additives (MIX) on CE activity in gilthead seabream (*Sparus aurata*) embryos.

Embryos were exposed from 24 to 96 hours post-fertilization to 0.1 and 1 mg/L BP extracts, as well as to 3 different concentrations of the MIX (0.1 nM, 0.01 and 1 µM). CE activity was assessed both *in vivo* and *in vitro* using a commercial recombinant human CE (hCE1) protein. In addition, the effects of individual compounds in the MIX on hCE1 activity were compiled from the literature.

In vivo exposure to BP extracts did not significantly alter CE activity, whereas the highest concentration of the MIX induced a significant increase. *In vitro* assays revealed greater sensitivity of CE activity to the MIX compared to BP extracts. A decline in CE activity was observed *in vitro* at 10 µM, with pronounced inhibition from 25 µM onwards. In contrast, individual compounds within MIX showed differential effects on hCE1 activity. Finally, BP extracts did not significantly affect CE activity *in vitro*.

Overall, these results indicate that mixtures of plastic additives can modulate CE activity during early development, potentially affecting CE-dependent processes such as xenobiotic and lipid metabolism. These findings highlight the importance of evaluating mixture toxicity and raise concerns about the sublethal effects of plastic during early-life stages.

P-037 - Environmental dopaminergic neurotoxicity induces parkinsonism like phenotypes in *Daphnia magna*

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Parkinson's disease (PD) is associated with dopaminergic neurodegeneration, in which mitochondrial dysfunction and oxidative stress play central roles. However, environmentally relevant invertebrate models for studying dopaminergic neurotoxicity remain limited. Here, we developed a Parkinsonism-like model in *Daphnia magna* using sublethal exposures to the dopaminergic neurotoxicants MPTP and rotenone. Both compounds induced concentration-dependent increases in reactive oxygen species, marked depletion of dopamine and related metabolites in the head, and a strong downregulation of key dopaminergic genes, indicating profound disruption of dopaminergic homeostasis and redox balance and supporting the construct validity of the model. These molecular alterations were accompanied by functional phenotypes supporting face validity, including reduced hopping distance, increased low-locomotion states, impaired photomotor responses and habituation, altered phototaxis and colour preference, particularly after MPTP exposure, and reduced heart rate. Together, these construct- and face-valid alterations support D. magna as a sensitive, integrative, and ecologically relevant model for investigating dopaminergic neurotoxicity and Parkinson's disease-related adverse outcome pathways triggered by environmental chemicals.

P-038 - Combined effects of microplastics and temperature on stress and metabolic responses in juvenile Pacu (*Piaractus mesopotamicus*)

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Introduction: Knowledge of the combined effects of multiple stressors on fish physiology remains limited. In aquaculture systems, environmental fluctuations, particularly temperature changes, can impair fish homeostasis and welfare. Among emerging contaminants, microplastics (MP) have attracted increasing attention due to their ubiquity in aquatic environments and their potential interactions with abiotic stressors. Extreme temperature events associated with climate change may exacerbate metabolic disturbances and oxidative imbalance in aquatic organisms, potentially amplifying the biological effects of contaminants. Therefore, this study investigated the effects of MP under different temperature conditions on the stress and metabolic responses of juvenile pacu (Piaractus mesopotamicus).

Materials and Methods: Juvenile pacu were exposed for 24 h to two experimental conditions: control (0 µg MP L^{-1}) and microplastics (20 µg MP L^{-1}), at three temperatures (16 °C, 28 °C, and 34 °C). The experiment consisted of 24 aquaria (four replicates per treatment), with two fish per aquarium (n= 48). After exposure, plasma cortisol and glucose levels were measured as stress indicators, while plasma triglycerides, cholesterol, and total serum proteins were analyzed as metabolic biomarkers.

Results and Discussion: After 24 h, cortisol levels were significantly elevated in fish maintained at 34 °C, regardless of MP exposure, whereas fish held at 16 °C exhibited the second highest values. The lowest cortisol concentrations were observed in fish maintained at 28 °C without MP exposure. These findings highlight temperature as a major physiological stressor in pacu, since both high (34 °C) and low (16 °C) temperatures activated the endocrine stress response. Cortisol is well-established biomarker of stress in fish and may undergo adaptive modulation according to the duration of the stressor exposure. Nevertheless, the elevated cortisol levels after 24 h suggest a sustained stress response under thermal challenge. In contrast, plasma glucose, triglycerides, cholesterol, and total protein concentrations did not differ significantly among treatments, suggesting that the 24 h exposure to MPs and temperature variation did not induce detectable metabolic disturbances. Furthermore, the absence of significant MP effects may indicate that, under the tested conditions, temperature exerted a more pronounced physiological influence than the contaminant.

Conclusion: This study demonstrates the direct influence of temperature on physiological stress responses in juvenile pacu, reinforcing thermal variation as a critical environmental stressor in aquaculture and aquatic ecosystems. Although MP exposure did not induce significant metabolic alterations within the 24 h exposure period, potential interactive effects between MPs and temperature warrants further investigation. Studies involving longer exposure periods and different

contamination scenarios are needed to clarify the combined impacts of these stressors on fish physiology, metabolism and health.

This project was funded by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico; grant # 176148/2023-0) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo; grants # 2024/19330-5, 2024/13478-0).

P-039 - Adipose tissue as an endocrine organ: recent insights into the anatomy and phylogeny of fat storage and regulation

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Fatty tissue is a site of lipid and energy storage and exerts manifold anatomical and mechanical functions. During the last decades, adipose tissue has been increasingly recognised to be an active synthesis site, particularly as a source of cytokines, growth factors and hormones involved in the complex orchestration of appetite and nutrition. In this morphological comparative study, we summarise recent insights into the anatomy and histology of adipose tissue as an endocrine organ, particularly as a source of cytokines, growth factors and hormones involved in the complex orchestration of appetite and nutrition. Special emphasis is laid on the ontogeny and phylogeny of fat storage in vertebrates. We further compare underlying mechanisms of endocrine metabolism, particularly during phases of growth and reproduction and during different environmental adaptations in vertebrates ranging from fish to mammals.

P-040 - Sentinels of the Conero: The Wild "Mosciolo" of Portonovo as a place-based education model for climate change awareness in schools

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Global climate change represents a critical threat to coastal marine ecosystems, driving severe impacts such as marine heatwaves, mucilage formation, and seawater acidification due to increased carbon dioxide (CO2CO2) absorption. These anthropogenic stressors jeopardize the survival and maintenance of numerous marine organisms. Within this context, the wild mosciolo of Portonovo (*Mytilus galloprovincialis*)—a unique population that reproduces spontaneously on the rocky cliffs of the Conero promontory (Ancona, Italy)—is highly vulnerable. Its habitat, the Adriatic Sea, is a semi-enclosed basin exposed to severe environmental degradation and historical overexploitation, which culminated in a total fishing ban during the 2025 summer season. Consequently, this resource, which is both a Slow Food Presidium and a key socio-economic and cultural symbol for the Ancona coast, requires urgent conservation strategies.To address this ecological crisis through educational interventions, the project "Fuori dal guscio! Custodiamo il mare, nutriamo il sapere, seminiamo il futuro" was funded by Cariverona. The initiative engaged secondary school students, including those from hospitality institutes, in educational workshops designed to raise awareness about the threats to wild mussel stocks. The core objectives included supporting natural population recovery and assessing the economic and sustainable value of local mussel farming. Additionally, to enrich the students' vision of sustainability and circular economy, the program integrated the study of innovative systems such as hydroponics for zero-km vegetable production. This multidisciplinary approach demonstrates how sustainable marine resource management, coastal biodiversity conservation, and agri-food innovation can collectively enhance territorial resilience. The project aims to measurably increase environmental awareness and ecological responsibility regarding food systems among participants, while strengthening technical knowledge of coastal ecosystems and sustainable supply chains. Beyond its educational outcomes, the project is structured to generate a significant regional impact: first, by fostering community- based participatory conservation of the wild mosciolo; second, by enhancing the visibility of local mussel farmers as active custodians of coastal sustainability. In conclusion, the 'Fuori dal guscio!' project highlights how synergies between the research community, educational institution, local production sectors, and civil society provide a powerful framework for climate change education. Connecting the vulnerability of a local identity symbol like the mosciolo to sustainable innovation and gastronomic enhancement represents an effective action strategy to stimulate the active engagement of young people, thereby empowering conscious citizens capable of serving as active custodians of biodiversity within the Mediterranean basin.

P-041 - Changes in the expression level of the gene encoding short Neuropeptide F in the beetle *Tenebrio molitor* in Response to environmental stress

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Rhythmic changes in physiological and behavioral processes are observed in living organisms in response to environmental factors such as light, temperature, and food availability. One of the regulatory molecules involved in the regulation of physiological processes in insects is short neuropeptide F (sNPF). This neuropeptide exhibits pleiotropic activity and is involved, among others, in the regulation of feeding, growth, and foraging behavior, but interspecies differences in the mode of sNPF action have been reported.

This study aimed to analyze changes in the expression level of the gene encoding sNPF precursor in the beetle *Tenebrio molitor* in response to environmental stress factors, including starvation and altered light conditions. The analyses were conducted in two tissues of the neuroendocrine system, the brain and the ventral nerve cord, to evaluate the relationship between sNPF precursor gene expression, starvation status, and photoperiod conditions. Changes in the expression level of the sNPF-encoding gene were assessed in *T. molitor* larvae subjected to starvation for 7, 14, and 21 days. In addition, the influence of different light regimes on gene expression was determined in adult beetles exposed to the following photoperiods: 24 h light, 24 h darkness, 12 h light:12 h darkness, and 8 h light:16 h darkness.

The results demonstrated that sNPF is involved in the regulation of feeding-related mechanisms in beetles and that changes in the expression level of the sNPF-encoding gene in the brain and ventral nerve cord are associated with the starvation state of insects. Furthermore, the expression level of the sNPF gene was shown to depend on light conditions, suggesting a possible role of this neuropeptide in the regulation of circadian rhythms in *T. molitor*. These findings may contribute to a better understanding of the role of sNPF in the regulation of insect metabolic and behavioral processes, as well as mechanisms of adaptation to changing environmental conditions.

P-042 - Computational reconstruction and prioritization of neuropeptide receptor candidates in *Tenebrio molitor*

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Neuropeptide signaling systems in insects remain incompletely characterized outside a limited number of model organisms, despite their central role in physiological regulation, stress adaptation, metabolism, feeding, and neuroendocrine coordination. Indeed, among Coleoptera several predicted neuropeptide receptors are poorly annotated or unresolved due to fragmented annotations, inconsistent transcript-protein mappings, and limited experimentally receptor validation. Here, we developed an integrative computational framework for large-scale identification and prioritization of neuropeptide receptor candidates in the mealworm *T. molitor* (Tenebrionidae), with particular focus on the HanSolin peptidergic system. A curated receptor atlas was reconstructed using a workflow combining genomic, transcriptomic and proteomic data followed by GPCR-focused candidate filtering, transmembrane topology prediction, domain annotation, comparative homology analysis, ligand-feature-guided receptor prioritization, and structural docking-based evaluation.Subsequently, candidate receptors were prioritized through multi-layer evidence integration, including sequence similarity to known insect neuropeptide receptors, GPCR structural characteristics, predicted transmembrane organization, conserved domains, and peptide–receptor compatibility. Through the integrative pipeline, we reconstructed a curated set of approximately 49 high- confidence neuropeptide receptor candidates representing multiple established insect neuropeptide signaling systems, including receptors associated with conserved peptide families as well as less-characterized candidates such as allatostatin A and HanSolin-related receptors. Notably, the analysis identified a candidate allatostatin A-like signaling component in *T. molitor*, despite previous reports suggesting lineage-specific losses or extensive modification of allatostatin A signaling components in several Polyphagan beetle lineages. Comparative sequence analysis, GPCR-domain validation, and transcriptomic evidence collectively supported the biological plausibility of this receptor–ligand system and highlighted the utility of the framework for recovering previously unresolved neuropeptide signaling candidates in non-model insects.

Furthermore, structural modeling and peptide docking analyses were subsequently applied to shortlisted HanSolinreceptor candidates to evaluate potential interaction compatibility and binding-site plausibility. The developed framework enabled refinement of receptor annotations, reduction of false-positive GPCR assignments, and recovery of biologically plausible HanSolin-associated receptor candidates from a large-scale *T. molitor* receptor search space. The workflow additionally provides a scalable strategy for neuropeptide receptor discovery in non-model insects where experimentally validated receptor datasets remain limited. Finally, this study establishes a reproducible receptor-prioritization pipeline integrating comparative endocrinology, bioinformatics, and structural modeling approaches for insect neuropeptide system deorphanization. The resulting receptor atlas and candidate prioritization strategy provide a foundation for future functional validation of neuropeptide signaling pathways in *T. molitor* and related beetle species.

P-043 - Inside the fly mind: A Strategy for spatial multiomics brain imaging to map neurochemical landscapes in *Drosophila melanogaster* by Imaging Mass Spectrometry

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Neuropeptides and biogenic amines are essential regulators of physiological processes and animal behaviour. Released from neurons, these signalling molecules show diverse structures, functions, and dynamic expression patterns in the nervous system. To unravel the complexity of neuronal communication, various techniques have been developed to detect and visualize signalling molecules in neuronal tissues. Monitoring the spatial and temporal distribution of these substances in the brain is essential for understanding how neuronal circuits process information and generate a coherent output. Imaging mass spectrometry has emerged as a powerful tool to chart neurochemical landscapes across the brain. However, its application to small and complex nervous systems remains challenging. In this study we show, how a matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI MSI) workflow was successfully applied to achieve high-resolution spatial mapping of neuropeptides in *Drosophila melanogaster* brain sections. Therefore, consecutive cryosections of wild-type fly brains were prepared and processed through washing and drying steps, followed by homogenous spray application of a matrix solution suitable for neuropeptide detection. The resulting MSI data were visualized as ion images showing the distribution and relative abundance of a specific molecule such as pigment-dispersing factor (PDF), which is a key circadian neuropeptide that synchronizes and modulates daily locomotor rhythms, across the sample surface. The spatial accuracy of the applied method becomes evident by comparing the neuropeptide derived ion maps with immunostainings. The results show that MALDI MSI enables label-free, *in situ* detection of biochemical compounds directly from the surface of neuronal tissue sections with the potential to identify previously uncharacterized peptides. Our workflow provides a basis for future investigations of state-dependent changes in neuropeptide levels in the brain of *D. melanogaster*.

P-044 - Long-term effects of intracerebroventricular administration of GnIH on energy homeostasis in male Japanese quail

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Gonadotropin-inhibitory hormone (GnIH), initially identified in Japanese quails, functions as an inhibitor of gonadal activity. GnIH neurons are located in the paraventricular nucleus and project to the median eminence and preoptic area. In addition, its role in feeding behavior has been proposed; acute intracerebroventricular (i.c.v.) administration of GnIH in chicks increases feeding behavior. These findings suggest that GnIH may regulate energy metabolism via the hypothalamus- pituitary axis. However, its long-term effects in birds remain unclear.

In this study, we performed chronic i.c.v. administration of GnIH to male quails for 13 days and analyzed food intake, body mass, organ masses, expression of genes related to feeding and lipid metabolism, and core body temperature. GnIH administration increased body mass without affecting food intake. The expression of feeding- and lipid metabolism-related genes was unchanged. Although the masses of subcutaneous and gizzard fat increased, liver mass decreased. Conversely, the masses of the heart, pancreas, kidney, testis, and both pectoralis major and minor muscles remained unchanged. In GnIH-treated quails, liver mass was negatively correlated with the expression of CPT1A, a gene encoding the rate-limiting enzyme of fatty acid β-oxidation. Core body temperature was unaffected.

These results indicate that long-term GnIH action may contribute to maintaining energy metabolic homeostasis, particularly lipid metabolism, in quails. Taken together, our findings demonstrate that brain-derived GnIH exerts long-term effects not only on reproductive suppression but also on the regulation of energy metabolism in male quails.

P-045 - Chronodisrupted feeding alters the responsiveness to ghrelin

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Feeding behaviour is not only a response to energetic demands but a rhythmic process coordinated by internal biological clocks. Predictable food availability acts as a potent *zeitgeber*, synchronizing metabolic, motivational, and hedonic components of feeding. When food timing becomes unpredictable, it induces chronodisruption, altering circadian organization and promoting anxiety-like states, with metabolic imbalance. Ghrelin, an orexigenic hormone with rhythmic secretion that peaks prior to expected feeding times, is a key integrator of homeostatic, hedonic, and circadian signals, and a plausible mediator of the physiological alterations induced by chronodisruption. This study examined whether feeding chronodisruption modifies physiological and behavioural responses to ghrelin in goldfish (*Carassius auratus*). To achieve this, fish were maintained for 5 weeks under a 12L:12D photoperiod (lights on at 8:00 h) and two feeding regimes: a fixed schedule (FS; daily at 10:00, ZT2) or a random schedule (RF; once daily at unpredictable times). Locomotor activity was continuously monitored to assess acclimation and circadian rhythmicity. Both groups maintained robust 24 h rhythmicity; however, only FS fish developed food anticipatory activity (FAA) as expected. RF fish lacked FAA and showed elevated overall activity, consistent with the loss of food arrival predictability. On day 22, ghrelin effect on anxiety-like behaviour and feed intake was assessed. All fish were fed at ZT2 and injected intraperitoneally with vehicle or ghrelin (10 pmol/g body weight), generating four groups: FS-Vehicle, RF-Vehicle, FS-Ghrelin, and RF-Ghrelin. One-hour post-injection, anxiety-behaviour of animals was evaluated by open field test followed by a 2-hour feed intake measurement. Both, maintenance under random feeding and ghrelin, independently increased thigmotaxis, indicating anxiogenic effects. The group with the combination of factors (RF-Ghrelin) displayed the highest anxiety levels, suggesting an additive effect of chronodisruption and ghrelin. Feed intake increased not only in ghrelin-treated fish but also in fish under RF conditions, whereas the combination of both factors did not increase the feed intake. On day 37, the ghrelin treatment was replicated maintaining the above mentioned four groups. Two hours post-injection fish were euthanized for transcriptional studies on hypothalamus, telencephalon, anterior intestine and liver. Chronodisruption markedly altered the neuroendocrine response to ghrelin. In the hypothalamus, ghrelin increased *npy* in FS fish but not in RF animals, the RF group showed elevated *npy* levels compared to control. Ghrelin upregulated orexin mRNA in FS fish but downregulated it in RF animals. Regarding anorexigenic peptides, the RF schedule and the ghrelin under FS reduced *pomca*, while *cartpt* decreased only in the RF-ghrelin group. Peripheral feeding regulators were also affected. Ghrelin treatment did not modify intestinal *ghrl* or hepatic *lepa1*, but RF fish showed reduced intestinal ghrelin and elevated hepatic leptin mRNA. Hedonic regulators exhibited strong schedule-dependent effects: telencephalic *cb1* increased with ghrelin, telencephalic *cb2* and hypothalamic *cb1* decreased under RF. Notably, overall data indicate that RF reduced dopamine-receptor mRNA levels, whereas ghrelin tended to enhance their expression in both schedules. Our findings show that feeding chronodisruption alters the coordination among orexigenic, anorexigenic, and hedonic pathways. Ghrelin acts as a

context-dependent modulator: it enhances anxiety-like responses, it preserves hedonic responsiveness and modulates intake-related signals depending on the feeding schedule of the animals. These results highlight temporal predictability as a key factor for emotional homeostasis and reinforce feeding-time regularity as a fundamental component of welfare in teleosts.

13. Miscellaneous

P-046 - Impact of combined exposure to nanoplastics and high temperatures on metabolic plasmatic markers in *Sparus aurata*

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In a world affected by emerging pollutants and climate change, the combined effects of nanoplastics and high temperatures on fish physiology remain still poorly understood. Here we analyzed the impact of chronic combined exposure to polystyrene nanoplastics (PS-NPs) and elevated temperatures on the metabolic plasmatic markers in *Sparus Aurata*, one of the economically valuable species in the Mediterranean region. Fish were placed in 30 ppt seawater with aged 40–46 nm PS-NPs at concentrations of 0 µg/L and 100 µg/L and exposed to 20 °C and 26 °C for 21 days, respectively. Blood was sampled at the end of the exposure period, and the supernatant from centrifugation was stored at −20 °C for analysis. 21 days after the exposure to single and combined stressors there were no significant changes in lactate, cholesterol, glucose or triglycerides among the groups. Plasma cortisol levels showed a remarkable increase in the combined PS-NPs+Temperature group, and, to a lesser degree, in the single stressor groups, indicating that individual stressors may also exert a moderate stimulatory effect on the endocrine stress axis of the juvenile *Sparus Aurata*. Plasma lysozyme levels, a sensitive marker of innate immune response, diminished in all the stressed groups. Even if the metabolic telltales analyzed did not change during sustained exposure to both stressors, the medium-term effects of cortisol and the decreased levels of lysozyme suggest an impaired response to inflammation resulting from the synergistic effects of combined PS-NPs and high-temperature exposure on the HPI axis, which may alter the welfare of S. aurata in the medium to long term.

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