aiec
ASOCIACIÓN IBÉRICA DE ENDOCRINOLOGÍA COMPARADA

BOOK OF ABSTRACTS

eman ta zabal zazu
Universidad del País Vasco Euskal Herriko Unibertsitatea
PiE
Plentziako Itsas Estazioa
Estación Marina de Plentzia
eman ta zabal zazu
Universidad del País Vasco Euskal Herriko Unibertsitatea
NAZIOARTEKO BIKAINTASUN CAMPUSA
CAMPUS DE EXCELENCIA INTERNACIONAL

ORGANIZING COMMITTEE

Ibon Cancio
Maren Ortiz Zarragoitia

Oihane Diaz de Cerio
Iratxe Rojo-Bartolomé
Ainara Valencia
Eider Bilbao
Beñat Zaldibar
Esther Blanco Rayon
Ane Rementeria
Tifanie Briaudeau
Joyanta Bir
Mar Santos
Evgeni Bunin
Anthony Nzioka Mutua
Miren P. Cajaraville
Xabier Lekube
Miren Bego Urrutia

AIEC BOARD

- **Isabel Navarro** (President), University of Barcelona
- **Jose Luis Soengas** (Vice-president), University of Vigo
- **Francisco Prat** (Secretary), Institute of Marine Sciences of Andalucía, Cadiz
- **Encarnación Capilla** (Treasurer), University of Barcelona
- **Mª Luz Pérez** (Vocal), Valencia Polytechnic University
- **Francisco Javier Sánchez-Vázquez** (Vocal), University of Murcia
- **Patricia Isabel Silvestre Pinto** (Vocal), Centre of Marine Sciences, Faro
- **Ibon Cancio** (Vocal), University of the Basque Country, Plentzia

ORGANIZERS AND SUPPORT

Universidad del País Vasco · Euskal Herriko Unibertsitatea

PiE Plentziako Itsas Estazioa · Estación Marina de Plentzia

aiec Asociación Ibérica de Endocrinología Comparada

Universidad del País Vasco · Euskal Herriko Unibertsitatea

NAZIOARTEKO BIKAINTASUN CAMPUSA
CAMPUS DE EXCELENCIA INTERNACIONAL

hazi

LABOLAN

AIEC BOARD WELCOME ADDRESS

On behalf of the Iberian Association for Comparative Endocrinology (AIEC) I welcome all the participants to the XIV AIEC congress. A new edition of the AIEC meetings begins now, after our last get-together online meeting in 2021, what means that fortunately, we can meet again face to face after four long years, in which many things have happened including, as you well know, a pandemic. For this reason, we are encouraged, more than ever, to initiate this meeting, at this time, here in Bilbao.

We do not want to miss the opportunity to declare again that our Society aims to continue promoting the participation of training students and young researchers in its conferences, within a framework of respect and cordiality. Another objective is to fortify the scientific collaborations between the different groups belonging to the Society, as well as incorporating new scientists and research groups to the AIEC family. This congress is once more, a preferential time for learning and networking.

We are sure that all the participants will contribute to the successful development of the meeting with the presentations of their investigations that will extend from fundamental questions in endocrinology to the most applied research in different related areas with the use of distinct approaches ranging from the molecular to the environmental levels.

In conclusion, this XIV AIEC congress is an excellent opportunity to share and update our latest advances in the scientific fields encouraged by the Society, but also in our lives, as always do the good friends when they meet again.

Welcome to Bilbao and the XIV AIEC meeting!

Isabel Navarro
President of the AIEC

WELCOME ADDRESS BY LOCAL ORGANIZING COMMITTEE

Zorionekuak gu!!

"*Zorionak*" is the Basque word to say congratulations, but that we can also use to say that we are fortunate. We are fortunate to have you among us in Bilbao. Etymologically the word may come from "*Txori*" = bird and "*ona*" = good, meaning that the good birds following their migrating "rhythms" and "environmental fluctuations" come to us in spring, bringing life, prosperity and productivity. Last year, "*Sorioneku*" was the word in the mouth of everybody in this part of the world. It was the only decipherable word written on the "*Hand of Irulegi*", a bronze piece found in the Iron Age archeological site of Irulegi (Navarre) dating the use of our "communication" language 2100 years back. Endocrinology is about communication in metazoans, cell to cell communication, and different organisms do it differently. In this congress, we shall communicate among ourselves using all the languages of the Iberian Peninsula and some more, because we are communicating beings! That is precisely the role on (neuro)endocrine systems. Communication is Power!!

The AIEC congress is normally a small gathering of friends and this one is not different. AIEC2023 is backboned around five thematic sessions. We shall talk about our daily academic and scientific "rhythms", the quality of "food" during the congress and of course about the weather and how the "environment is changing" and affecting us. Those of us with some history on our shoulders will talk with old friends, that we only see from congress to congress. Some will talk about their children, biological or scientific (that means that we shall talk indirectly about "reproduction") and how they are "growing". In any case, this congress should be a pulpit specially for young scientists and a learning arena to train their "congressing" abilities. In fact, we shall do a lot of collective "neuroendocrinology", we hope!

Take this as an invitation to enjoy the XIV AIEC 2023 congress, enjoy Bilbao and its surroundings, and let us hope that this will help to improve your "metabolism" and "immune system".

Ibon Cancio and Maren Ortiz-Zarragoitia
On behalf of the organizing committee

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ASOCIACIÓN IBÉRICA DE ENDOCRINOLOGÍA COMPARADA

PROGRAMME

MONDAY SEPTEMBER 11

13:00-15:00 REGISTRATION

15:00-15:30 WELCOME AND OPENING CEREMONY

15:30-16:30 OPENING LECTURE

Evelyn Houliston: Neuropeptidic regulation of oocyte maturation and spawning in the jellyfish *Clytia hemisphaerica*

SESSION REPRODUCTION

Chairpersons: Juan F. Asturiano and Oihane Diaz de Cerio

17:00-17:30. SESSION PLENARY

Sánchez-Baizán N. Variability in transcriptome dynamics of sex differentiation among vertebrates: exploring conserved pathways

17:30-17:45. ORAL 1

Orchard I. Glycoprotein hormone (GPA2/GPB5) and corticotropin-releasing factor-like diuretic hormone (CRF/DH) signaling pathways influence egg production in adult female *Rhodnius prolixus*

17:45-18:00. ORAL 2

Blanes-Garcia M. Cryopreservation and xenotransplantation of European eel (*Anguilla anguilla*) spermatogonial stem cells

18:00-18:15. ORAL 3

Salazar M. Epigenetic differences in the innate response after immune stimulation in gonads of zebrafish (*Danio rerio*)

18:15-18:30. ORAL 4

Ferrao L. Superoxidase dismutases (SODs) in the European eel

18:30-18:45. ORAL 5

Bir J. Analysis of yellowfin tuna *Thunnus albacares* ovarian maturation: molecular staging for the assessment of stock reproductive potential

19:00-20:00 WELCOME RECEPTION AND COCKTAIL

TUESDAY SEPTEMBER 12

08:30-09:00 REGISTRATION

SESSION NEUROENDOCRINOLOGY AND RHYTHMS

Chairpersons: Javier Sanchez and Encarni Capilla

09:00-09:30. SESSION PLENARY

Godino-Gimeno A. Circadian roles of the melanocortin system

09:30-09:45. ORAL 1

Herrera-Castillo L. Food anticipatory activity is an anxious condition in *Carassius auratus* that is mediated by ghrelin

09:45-10:00. ORAL 2

Barany A. Characterizing the peptidergic [ARG8]vasotocin system in the sea lamprey (*Petromyzon marinus*)

10:00-10:15. ORAL 3

Vergés-Castillo A. Light photoperiod and spectrum modulate daily rhythms of proliferation, apoptosis, DNA repair and oxidative stress markers in fish embryonic stem cells

10:15-10:30. ORAL 4

de Alba G. Daily rhythms of thermal preference in diurnal and nocturnal fish

10:30-10:45. ORAL 5

Knigge T. Antidepresants-the new endocine disruptors?

10:45-11:15 COFFEE BREAK

SESSION ENVIRONMENTAL ALTERATIONS AND BIOLOGICAL ANSWERS/IMPACTS

Chairpersons: Patricia I. Pinto and Francesc Piferrer

11:15-11:45. SESSION PLENARY

Pinto P. Time to act: protect the marine life from endocrine disrupting pollutants

11:45-12:00. ORAL 1

Schonemann A. *Cyprinodon variegatus* transcriptomic alterations caused by endocrine disruption compounds and compostable plastic bag lixiviates

12:00-12:15. ORAL 2

Mustapha UF. Can liquid tissues recapitulate epigenetic information in difficult-to-access internal tissues?

12:15-12:30. ORAL 3

James A. Towards a better consideration of endocrine disruption within the technical guidance for deriving environmental quality standards

12:30-12:45. ORAL 4

Castro L.F.C. The dolphin and the whale: from the past to the Anthropocene

12:45-13:00. ORAL 5

Cartan S. Non-invasive technology to detect stress biomarkers in fish

13:00-13:15. ORAL 6

Nzioka A. tracing life history of thicklip grey mullets inhabiting estuaries with different xenoestrogenic pressures along the Southern Bay of Biscay

13:00-14:45:LUNCH

SESSION GROWTH AND DEVELOPMENT

Chairpersons: Alicia Felip and Miguel M. Santos

14:45-15:15. SESSION PLENARY

Guerrero-Peña L. Morphological adaptations and gene expression patterns in flatfish eye migration: insights into visual system development and function.

15:15-15:30. ORAL 1

Sempere L. Growth and physiological performance of female European sea bass with different body size and gonadal development: comparison of RNA transcripts from liver tissue.

15:30-15:45. ORAL 2

Beato S. DNA methylation during early development in diploid and triploid European sea bass.

15:45-16:00. ORAL 3

Peng MX. Species-specific response of two marine bivalves to ocean acidification.

16:00-16:15. ORAL 4

Barros S. Toxicological risks of metformin for aquatic ecosystems: a generational study with zebrafish using environmentally relevant concentrations of metformin.

16:15-16:30. ORAL 5

Capela R. Embryo bioassays for toxicity testing: development of *Lymnaea stagnalis* embryo-tests for the hazard assessment of contaminants of emerging concern.

16:30-17:00 COFFE BREAK

17:00-18:00. PLANAS LECTURE

Miguel M Santos: Endocrine disrupting chemicals: from extrapolation to precision hazard assessment.

18:00-19:30. POSTER SESSION AND REFRESHMENTS

20:30-23:00 CONFERENCE DINNER:

Restaurant "Bascook" (https://www.bascook.com/) Barroeta Aldamar Kalea, 8, 48001 Bilbo

WEDNESDAY SEPTEMBER 13

SESSION FOOD INTAKE AND METABOLISM

Chairpersons: Isabel Navarro and Juan Miguel Mancera

09:00-09:30. SESSION PLENARY

Lourenço T. Estrogenic and statin impacts in lipid pathways of brown trout.

09:30-09:45. ORAL 1

Calo J. Dietary lipid sensing through fatty acid oxidation and chylomicron formation in gastrointestinal tract of rainbow trout.

09:45-10:00. ORAL 2

Godino-Gimeno A. Obesity impairs cognitive function with no effect on anxiety like behaviour in zebrafish.

10:00-10:15. ORAL 3

Leal E. Asprosin a potential role in the regulation of energy balance in fish.

10:15-10:30. ORAL 4

Comesaña S. Cortisol modulation of metabolic control of feed intake in rainbow trout hypothalamus.

10:30-10:45. ORAL 5

Chivite M. Impact of diets formulated with insect meal on the feeding behaviour of Atlantic salmon fry.

10:45-11:15 COFFE BREAK

11:15-12:15 CLOSING LECTURE

Maria Ina Armone: Gene regulatory networks meet cell type evolution: lessons from an 'urchin'.

12:15-13:15. GENERAL ASSEMBLY OF AIEC AND CLOSING CEREMONY

13:15-17:00. EXCURSION TO VISIT THE PLENTZIA MARINE STATION

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

	MONDAY	TUESDAY	WEDNESDAY
8:30		Registration	
8:45			
9:00		**Plenary** (NEUROENDO & RYTHMS): Godino-Gimeno et al	**Plenary** (FOOD INTAKE & METAB.) LourenÇo et al.,
9:15		Oral 1: Herrera-Castillo et al.,	Oral 1: Calo et al.,
9:30		Oral 2: Barany et al.,	Oral 2: Godino-Gimeno et al.,
9:45		Oral 3: Vergés-Castillo et al.,	Oral 3: Leal et al.,
10:00		Oral 4: de Alba et al.,	Oral 4: Comesaña et al.,
10:15		Oral 5: Knigee et al.,	Oral 5: Chivite et al.,
10:30			
10:45		Coffe break	Coffe break
11:00			
11:15		**Plenary** (ENVIR. ALTERATIONS). Pinto et al.,	**Closing Lecture. Maria Ina Arnone. GENE REGULATORY NETWORKS MEET CELL TYPE EVOLUTION:..**
11:30		Oral 1: Schonemann & Beiras	
11:45		Oral 2: Mustapha & Piferrer	
12:00		Oral 3: James et al.,	
12:15		Oral 4: Castro et al.,	**General Assembly of AIEC and closing ceremony**
12:30		Oral 5: Cartan et al.,	
12:45		Oral 6: Nzioka et al.,	
13:00	**Registration**		
13:15		Lunch with posters	
13:30			
13:45			
14:00			
14:15			
14:30			
14:45		**Plenary** (GROWTH & DEV.) Guerrero-Peña et al.	**Excursion to visit the Marine Station of Plentzia**
15:00	**Opening Ceremony**	Oral 1: Sempere et al.,	
15:15		Oral 2: Beato et al.,	
15:30	**Opening Lecture: Evelyn Houliston.** NEUROPEPTIDIC REGULATION OF OOCYTE…	Oral 3: Peng et al.,	
15:45		Oral 4. Barros et al.,	
16:00		Oral 5: Capela et al.	
16:15			
16:30	Coffe break	Coffe break	
16:45			
17:00	**Plenary** (REPRODUCTION). N Sanchez-Baizan et al.,	**Planas Lecture: Miguel M Santos.** ENDOCRINE DISRUPTING CHEMICALS: FROM EXTRAPOLATION TO…	
17:15			
17:30	Oral 1: Orchard et al.,		
17:45	Oral 2: Chen et al.,		
18:00	Oral 3: Salazar et al.,	**Poster session** & refreshments	
18:15	Oral 4: Ferrao et al.,		
18:30	Oral 5: Bir et al.,		
18:45			
19:00	**Welcome reception & COCKTAIL**		
19:15			
19:30			
19:45			
20:00			
20:15			
20:30		**CONFERENCE DINNER**	
20:45			
21:00			
21:15			
23:00			

COMMUNICATIONS

PLENARY LECTURES

AIEC is pleased to announce that the “Planas Lecture” that is delivered in every AIEC congress by an eminent invited endocrinologist in honor of Professor Josep Planas Mestres, a pioneer Iberian scientist in comparative endocrinology, has been awarded to Professor Miguel Santos. This lecture will take place on the 12th of September during the second day of the congress.

PLANAS LECTURE 2023

Prof. Miguel Santos

University of Porto, Interdisciplinary Centre for Marine and Environmental Research (CIIMAR)

Lecture title: **Endocrine disrupting chemicals: from extrapolation to precision hazard assessment**

Short Bio:

Miguel Santos is a professor at the Dept. of Biology of the Faculty of Sciences University of Porto and a researcher at the Interdisciplinary Centre for Marine and Environmental Research (CIIMAR) of the University of Porto where he coordinates the group of “Endocrine Disruptors and Emergent Contaminants, https://www2.ciimar.up.pt/research.php?team=10”. His main focus of research has been improving hazard and risk assessment of Endocrine Disrupting Chemicals (EDCs) and Contaminants of Emerging Concern (CECs) in aquatic ecosystems in a climate change scenario, while at the same time developing new tools to address their effects. He uses molecular biology, biochemistry and bioinformatics to better understand the mode of action of EDCs and CECs; to link this mechanistic approach with the outcomes at individual levels he uses ecotoxicity tests with a large diversity of taxa. Ecological modeling is used to extrapolate individual-collected data of ecotoxicological studies and/or field data to an ecological-relevant level. All research is articulated with legal instruments such as WFD, MSFD, REACH.

INVITED PLENARIES (Opening and Closing lectures)

Three will be two invited plenary lectures in the AIEC2023 congress, one will be delivered as the opening lecture of the 11th of September and the other one as the closing lecture on the 13th, by eminent researchers doing rocket science on model organisms.

OPENING LECTURE

Evelyn Houliston

Villefranche-sur-mer Developmental Biology Laboratory, CNRS/Sorbonne University, IMEV (France)

Lecture Title: **Neuropeptidic regulation of oocyte maturation and spawning in the jellyfish *Clytia hemisphaerica***

Short Bio:

Evelyn Houliston is a developmental cell biologist by training. During her PhD (University of Cambridge UK) and successive post-docs (Institut Jacques Monod, Paris, then University of Toronto, then the Villefranche-sur-mer marine station) she addressed a variety of developmental events at the cellular level: Compaction in the mouse embryo, Cortical Rotation in the amphibian egg, Axis specification in the ctenophore *Beroe*, Surface Contraction Waves in *Xenopus* embryos, and maternal mRNA localisation in amphibians and cnidarians. Motivated by the diversity and practical advantages of marine invertebrate embryos for imaging and embryology, she moved to the Developmental Biology Laboratory in Villefranche–sur-mer (LBDV) where was recruited as a CNRS staff researcher in 1993 and established an independent group in 1995. One of her principal scientific contributions has been to identify and develop with her group *Clytia hemisphaerica* as a new model species for cell, developmental and evolutionary studies. From 2009 to 2018 she was Director of the LBDV and in 2021 she was elected EMBO member.

CLOSING LECTURE

Maria Ina Arnone

Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples (Italy)

Lecture Title: **Gene regulatory networks meet cell type evolution: lessons from an 'urchin'**

Short Bio:

Maria Ina Arnone, biochemist by training, is a developmental molecular biologist with expertise in gene expression analysis, functional genomics and gene regulatory network (GRN) studies. After a period of three years (1995-1998) at the California Institute of Technology, Pasadena, CA, where she contributed to a seminal work on the organization and function of genomic regulatory systems, she established her group at Stazione Zoologica in Naples with the aim of studying evolution of organs and body parts by comparison of the GRNs that control the formation of such parts in different animals. Using the sea urchin embryo as main model system, she recently developed a novel approach integrating various 'omics' technologies, including single cell/nucleus transcriptomics and chromatin accessibility measurements, to study developmental GRNs and their evolution. Currently research director at Stazione Zoologica Anton Dohrn, she was elected EMBO member in 2018.

OPENING LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

NEUROPEPTIDIC REGULATION OF OOCYTE MATURATION AND SPAWNING IN THE JELLYFISH CLYTIA HEMISPHAERICA

Houliston E *.

Laboratoire de Biologie du Développement de Villefranche-sur-mer (LBDV), IMEV, UMR7009 Sorbonne Université/CNRS, 06230 Villefranche-sur-mer, France. Email : houliston@imev-mer.fr

In the hydrozoan jellyfish *Clytia hemisphaerica,* gametes are released every day upon a dark-light cue. Using this convenient laboratory species we have identified key molecules that mediate light-triggered spawning. We first identified a small neuropeptidic Maturation Inducing Hormone (MIH) secreted by specialised neurosecretory cells in the gonad ectoderm in response to light. MIH consists of PRP/PRA/PRYamide type amidated tetrapeptides, synthesized from two distinct precursor polypeptides. MIH provokes spawning in both males and females at nanomolar concentrations, and so may contribute to spawning synchronisation between animals. We found that the MIH cells also express an opsin photopigment, *Clytia* Opsin9. Opsin9 mutant jellyfish generated using CRISPR/Cas9 failed to spawn, a phenotype reversible by MIH. Finally, we identified an oocyte-expressed GPCR able to bind MIH as the MIH Receptor (MIHR). *Clytia* jellyfish from *MIHR* mutant lines show defects in gamete development and spawning. Oocytes from these mutants fail to mature is response to synthetic MIH. but responded to treatment with cAMP analogues. Thus in female *Clytia*, Opsin9 mediated release of MIH in the morning leads to meiotic maturation and subsequent spawning via binding to the MIHR on the oocyte surface and signaling via $G\alpha_S$, cAMP and PKA. Molecular phylogeny grouped the *Clytia* MIHR with a subset of bilaterian neuropeptide receptors including Neuropeptide Y, Gonadotropin Inhibitory Hormone, pyroglutamylated RFamide and Luqin, shedding light on the evolution of neuropeptide-hormone systems.

Acknowledgements. Most of this work was done by Gonzalo Quiroga-Artigas during his PhD within the EU ITN "NEPTUNE". This involved close collaboration with N. Takeda (Tohoku University) and R. Deguchi (Miyagi University of Education) for MIH identification, and with G. Jekely and P. Bauknecht (MPI Tübingen) for GPCR deorphanisation. T. Momose, L. Leclère, J. Uveira, S. Chevalier and P. Lapébie (LBDV) also made important contributions.

PLANAS LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ENDOCRINE DISRUPTING CHEMICALS: FROM EXTRAPOLATION TO PRECISION HAZARD ASSESSMENT

Santos MM[1, 2]*.

[1]*FCUP – Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre S/N, 4169-007 Porto, Portugal. [2]CIIMAR/CIMAR-LA – Interdisciplinary Centre of Marine and Environmental Research, Avenida General Norton de Matos S/N, 4450-208 Matosinhos, Portugal. *Email: miguel.santos@fc.up.pt

Currently over 100,000 chemicals are continuously produced, with many ultimately reaching aquatic ecosystems. Some of these compounds can interfere with animal physiology, as they show the ability to mimic or block the function of endogenous hormones or signaling pathways, thus representing a major threat to ecosystem health. These compounds, commonly termed endocrine disrupting chemicals (EDCs), display a wide array of structures and can be bioactive at rather low levels, particularly in sensitive time-windows such as embryonic development and sex differentiation. Given the enormous diversity of endocrine systems among metazoans, if we aim to protect biodiversity at an ecosystem scale, understanding the EDCs mode of action (MoA) in disparate taxa should be a central piece of toxicity testing and hazard assessment. In fact, several examples indisputably demonstrate that the genomic constitution of a given species is a key aspect that determines its response towards chemical insults. Understanding the MoA of environmental chemicals in a wide array of taxa, allows the building of a toxicant response, thus supporting an improved hazard and risk assessment. This approach is today possible given the increase of information on full genome and transcriptome projects bringing an unprecedent tool for addressing the hazard assessment of EDCs, both in invertebrates and vertebrates. Importantly, recent evidences indicate that an increasing number of environmental chemicals may disrupt development and other endpoints not only in the parental exposed generation, but across multiple subsequent non-exposed generations, through modulation of the epigenome. These findings have major implications for understanding disease etiology and adverse outcomes. Here, we will discuss the risks of EDCs framed in current hazard and risk assessment frameworks, and explore major challenges in the field.

Acknowledgements. ATLANTIDA – Platform for the monitoring of the North Atlantic ocean and tools for the sustainable exploitation of marine resources" with the reference [NORTE-01-0145-FEDER-000040] financed by "Comissão de Coordenação e Desenvolvimento Regional do Norte (NORTE2020)" through Portugal 2020 and "Fundo Europeu de Desenvolvimento Regional (FEDER).

CLOSING LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

GENE REGULATORY NETWORKS MEET CELL TYPE EVOLUTION: LESSONS FROM AN 'URCHIN'

Arnone MI.

Stazione Zoologica Anton Dohrn (SZN), Villa Comunale, 80121, Napoli, Italy.

One intriguing and still open fundamental question in biology is how different embryonic structures or distinct organs, originating from the same embryonic tissue, developed and evolved in different animals. The answer to these questions most likely lies in the complex nature of the developmental gene regulatory networks (GRNs) that functionally connect transcription factors (TFs) to the terminal differentiation genes of a specific cell type. By using a combination of different omics methods, including information on the chromatin availability, as well as gene expression profiles of individual cells, tissues and embryos, we were able to uncover the components of developmental GRNs and their interactions in the sea urchin larva at unprecedented resolution. Focusing on pancreas evolution, we found distinct cell type families with a strong pancreatic-like signature in the sea urchin feeding larva, the stage when this little animal of a few thousands cells begins its planktonic life in the water column where it is exposed to and interact with a complex environment. Overall our data indicate i) the presence of an endocrine pancreas network operating in a subset of sea urchin neurons that was probably active in an ancestral cell type and then inherited by neuronal and pancreatic developmental lineages in sea urchins and vertebrates ii) that the exocrine cells found in the midgut of the sea urchin larva resemble both transcriptomically and morphologically the acinar cells of mammals, suggesting that these building blocks of the mammalian pancreas are already present and operating in this “pancreas-less” organism.

SESSION 1.
REPRODUCTION

PLENARY LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

VARIABILITY IN TRASNCRIPTOME DYNAMICS OF SEX DIFFERENTIATION AMONG VERTEBRATES: EXPLORING CONSERVED PATHWAYS

Sánchez-Baizán N[1]*, Jarne-Sanz I[1], Roco AS[2,3], Schartl M[2,4], Piferrer F[1].

[1]*Institut de Ciències del Mar (ICM), Spanish National Research Council (CSIC), Barcelona, Spain. [2]Developmental Biochemistry, Biocenter, University of Wuerzburg, 97074 Wuerzburg, Germany. [3]Department of Experimental Biology, Faculty of Experimental Sciences, University of Jaén, Campus Las Lagunillas S/N, 23071 Jaén, Spain. [4]Xiphophorus Genetic Stock Center, Texas State University, San Marcos, TX 78666, USA.

*Email: nsbaizan@icm.csic.es

Gonadal sex differentiation is a complex process involving dimorphic expression of many genes, initially thought to be relatively conserved across vertebrates. Here, we characterized gene expression dynamics and quantified the proportion of genes activated and repressed in each sex of six vertebrate species, from fish to mammals. Our results unambiguously show an extraordinary variability across species, with activation/repression involving between 8% and 92% of the male- and female-enriched genes in one sex or the other depending on the species but without a relationship with the position in the vertebrate phylogenetic tree. Furthermore, key genes, defined as genes previously known to be involved in sex development, such as dmrt1, sox9 and amh, if enriched, were consistently enriched only in males, whereas key genes such as foxl2, cyp19a1a and fst, if enriched, were only enriched in females. Of note, and in contrast to key genes, the top 10% most expressed genes were not always enriched in the same sex and included 16 potential novel markers of early sex differentiation. Moreover, we observed that the genes involved in the process might be more variable than previously thought. Thus, we propose that gonadal sex differentiation in vertebrates can be seen as a hierarchical network with hub genes such as sox9 and amh and less connected nodes involving genes such as nr3c1 and hoxd10. In such a scenario, evolutionary pressures may affect genes depending on the number of connections. However, whether those genes belong to the same biological processes or pathways remains unknown (i.e., if the pathways are conserved). Therefore, we will also present, for the first time, the functional enrichment analysis (GO terms and pathways) of each species separately and discuss the similarities and differences found across them.

Acknowledgements. Research supported by Spanish Ministry of Science grant no. PID2019-108888RB-I00 to FP.

ORAL COMMUNICATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

GLYCOPROTEIN HORMONE (GPA2/GPB5) AND CORTICOTROPIN-RELEASING FACTOR –LIKE DIURETIC HORMONE (CRF/DH) SIGNALING PATHWAYS INFLUENCE EGG PRODUCTION IN ADULT FEMALE *RHODNIUS PROLIXUS*

Orchard I*, Al-Dailami A, Lange AB.

University of Toronto Mississauga, Department of Biology, 3359 Mississauga Rd., Mississauga, ON, Canada L5L 1C6. *Email: Ian.orchard@utoronto.ca

Glycoprotein hormones are formed by the heterodimerization of alpha and beta subunits. In vertebrates, there are five glycoprotein hormones, four of which have a common alpha subunit (GPA1) bound to a specific beta subunit (GPB1, GPB2, GPB3, or GPB4), while the fifth, thyrostimulin, which is also found in invertebrates, is formed by the dimerization of GPA2 and GPB5 subunits. The mammalian corticotropin-releasing factor (CRF) is involved in stress responses, and in insects can act as a diuretic hormone (CRF/DH). Previous reports on invertebrates suggest that GPA2/GPB5 and CRF/DH play critical roles in feeding, diuresis, and reproduction. Here we show that GPB5 and CRF/DH are co-localized in lateral neurosecretory cells of the mesothoracic ganglionic mass and their abdominal nerve neurohemal sites in the blood gorging vector, *Rhodnius prolixus*. qPCR reveals that the GPA2/GPB5 receptor (LGR1) and the CRF/DH receptor (CRF/DHR) transcripts are expressed in ovaries and fat body in adult female *R. prolixus*, and their expression levels increase post-blood meal; a stimulus that triggers diuresis and reproduction. To examine the involvement of the GPA2/GPB5 and CRF/DH signaling pathways in egg production, transcript expression of LGR1 and CRF/DHR was knocked down using dsRNA and egg production monitored by examining the production of the major yolk protein, vitellogenin (Vg), the number and quality of eggs laid, and their hatching rate. The results suggest that these two neurohormones are released at feeding to delay egg production.

Acknowledgements. This work is supported by NSERC discovery grants to ABL and IO.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SOMATOSTATIN SIGNALLING REGULATES FECUNDITY AND METABOLISM IN ZEBRAFISH

Chen J[1,2]*, Zhao W[2], Cao L[2], Martins RST[2], Canário AVM[1,2].

[1]*CCMAR Centro de Ciências do Mar, Universidade do Algarve, Portugal. [2]*Ocean University, Shanghai, China. *Email: a63571@ualg.pt

Energy allocation between growth and reproduction determines puberty onset and fertility. In mammals, peripheral hormones, such as leptin, insulin and ghrelin, convey metabolic signals to the central nervous system to activate gonadotrophin-releasing hormone neurons and trigger puberty. However, the regulatory signals and mechanisms integrating metabolism and reproduction are still largely unknown, especially in fish. Here we report the phenotype of CRIPSR/Cas9 edited mutant lines *somatostatin 1* (*ss1*) and *somatostatin 3* (*ss3*). Both *ss1 and ss3* mutants showed 20-30% over-proliferation of primordial germ cells, a process mediated by *sstr2a* receptor signalling. However, only the adult *ss3* mutant (not the *ss1* mutant) laid 50% more eggs than their wild type controls. The *ss1* mutant line were hyperglycaemic, with decreased glucose tolerance, higher triglyceride levels and increased pancreatic α-cell proliferation compared to the wild type. In contrast, the *ss3* mutants showed hypoglycaemia, increased glucose tolerance, lower triglyceride and total cholesterol together with increased pancreatic β-cell proliferation compared to the wild type. Our results demonstrated for the first time that somatostatin signalling tightly controls energy and fecundity through its inhibitory role on pancreatic cells and PGCs proliferation. This is likely to be a general function of somatostatin in vertebrates.

Acknowledgements. This work was supported by funds provided the by the Shanghai Municipal Government to Shanghai Ocean University and by the FCT - Foundation for Science and Technology through projects 2022.08828.PTDC, UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 to CCMAR.

WITHDRAWN FOR VISA ISSUES

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CRYOPRESERVATION AND XENOTRANSPLANTATION OF EUROPEAN EEL (*Anguilla anguilla)* SPERMATOGONIAL STEM CELLS

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

[1]*Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València. Camino de Vera s/n, 46022. Valencia, Spain.[2]Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences. Páter Károly u. 1, 2100, Gödöllő, Hungary. [3]Laboratoire Service d'Experimentations Aquacoles, IFREMER, INRAE. Station de Palavas, Chemin de Maguelone 34250, Palavas-les-Flots, France. *Email: marblaga@posgrado.upv.es

European eel faces reproductive challenges leading to its population decline. Hormonal treatments for eel reproduction in captivity induce variable gamete quality, hence we are attempting to develop surrogate broodstock technology for their conservation. This study aimed to cryopreserve eel spermatogonial stem cells (SSCs) and evaluate their functionality through xenotransplantation into common carp. Immature eel testes were dissected to develop SSC preservation protocols using slow-rate freezing, vitrification, and hypothermic storage. Six cryoprotectants supplemented with sugars and proteins were tested during freezing. The optimal cryomedium consisted of 1.5 M DMSO, 0.1 M glucose, and 1.5% BSA (50% viability). During vitrification we tested three different equilibration, vitrification, and warming solutions. Using propylene glycol and DMSO yielded a viability around 70%. Hypothermic storage (4 ºC) in L-15 medium induced higher viability in cell suspensions (75% after 144 hours) compared to whole tissue. Thawed SSCs were stained with PKH-26 and microinjected into *dnd1*-knockdown carp larvae. After 1.5 months post-transplantation (mpt), fluorescent cells were observed in the gonads of 8 out of 25 carps. However, at 6 mpt, qPCR analyses did not show eel-specific sequences for *vasa* and *dnd1* genes in 14 sampled common carps, and histological examination revealed no gonadal development in the sterilized recipient carps. In conclusion, we successfully cryopreserved European eel SSCs, but common carp did not provide a suitable gonadal microenvironment for eel SSCs development. Currently, we are testing zebrafish and European sea bass as potential alternative recipients.

Acknowledgements. Supported by MCIU (EELGONIA; RTI2018-096413-B-I00), European Union NextGenerationEU (PRTR-C17.I1) and Generalitat Valenciana (THINKINAZUL/2021/012) to SEASPERM. Partially funded by EU H2020 Research Innovation Program (AQUAEXCEL3.0; grant agreement No. 871108). MBG and LF have PhD grants from UPV (PAID-01_20) and GVA (GRISOLIAP/2020/063), respectively. ZM and ÁH were supported by the NKFIH K138425 and FK 142933 projects.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

EPIGENETIC DIFFERENCES IN THE INNATE RESPONSE AFTER IMMUNE STIMULATION IN GONADS OF ZEBRAFISH (*DANIO RERIO*)

Salazar M[1]*, Esteve A[2], Joly S[1], Ribas L.[1]

[1]*Institut de Ciències del Mar, Spanish National Research Council (CSIC), Passeig Marítim de la Barceloneta, 37–49, 08003, Barcelona, Spain. [2]Centre Nacional d'Anàlisi Genòmica (CNAG-CRG), Centre de Regulació Genòmica, Parc Científic de Barcelona – Torre I, Baldiri Reixac 4-8, 08028 Barcelona. *Email: marcesm27@gmail.com

Epigenetics has gained significant attention in recent years due to its pivotal role in various biological processes. In fish, epigenetic modifications have been recognized as key regulators of development, growth, and adaptation to environmental factors. Interestingly, emerging evidence suggested that epigenetic mechanisms may also play a crucial role in modulating the response of the fish gonads to infectious agents. In fact, gonadal factors, including reproductive hormones and cytokines, can modulate immune cell activities, regulate the production of immune molecules, and influence the overall immune response. Although the existence of the interaction reproductive-immune system is well-known, the underlying epigenetics mechanisms needs further elucidation. To study the epigenetic mechanisms by the DNA methylation patterns of the gonadal immune response, ovaries and testis of adult zebrafish (*Danio rerio*) were immune-stimulated by an intraperitoneal injection of lipopolysaccharides (LPS). The DNA methylation of two relevant innate immune genes (*Il1β* and *Casp9*) were studied by a candidate gene approach at a single nucleotide resolution by sequencing strategies. The average methylation difference of the *Casp9* gene was significant for the interaction of treatment and sex, whereas for the *Il1β* gene average methylation difference only sex was significant. Additionally, we observed the existence of a DNA methylation differences in the epigenetic response between sex and infection in the *Casp9* gene, an observation that was depending on the CpG locus. The data suggested the existence of interplay between sex and immune response in the fish gonads at the epigenetic level. Understanding these interactions is crucial for unraveling the mechanisms underlying sexually dimorphic immune responses in reproductive tissues.

Acknowledgments. This study was supported by the Spanish Ministry of Science and Innovation grant 2PID2020-113781RB-I00 "MicroMet" and by the Consejo Superior de Investigaciones Científicas (CSIC) grant 02030E004 "Interomics" to LR.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SUPEROXIDASE DISMUTASES (SODs) IN THE EUROPEAN EEL

Ferrão L*, Blanes-García M, Pérez L, Asturiano JF, Morini M.

Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. 46022 Valencia. Spain. *E-mail: mlpirfer@posgrado.upv.es

Superoxide dismutases (SODs) are antioxidant enzymes that protect cells from excessive oxidation. Three SODs (SOD1-3) were identified in mammals but knowledge in teleost is limited. The study aimed to characterize the SODs in the European eel and describe their expression patterns during eel testis maturation. First, we analysed the European eel genome through BLAST analyses to identify SODs sequences. Phylogenetic and synteny analyses were performed to study SODs evolution in vertebrates. The presence of one SOD1, and two SOD2/3 (a and b) in the eel indicated a SOD2 and SOD3 duplication in elopomorphs. Once identified, SODs expression levels were measured in various tissues of males and females. SOD1 exhibited higher expression in females compared to males in all tissues, while SOD2a/b were highly expressed in brain-pituitary-gonad axis tissues in both genders. SOD3a showed predominant expression in the ovary of females and the liver of males, while SOD3b was found in the pituitary and brain of both genders. Then, we studied the effects of different maturation protocols (standard hormonal treatment and cold seawater pre- treatment) on SODs expression during eel testis maturation. Salinity increase at the onset of standard treatment at 20 °C upregulated SOD1 and SOD2a/b at the beginning of spermatogenesis, while SOD3a/b expression remained unaffected. SOD2/3a decreased, while SOD2b increased through the spermatogenesis induced by the hormonal treatment. Pre-treatment with cold seawater (10 °C) downregulated SOD1 expression but increased SOD3a. Afterwards, the standard hormonal treatment downregulated SOD1 in eels without any pre-treatment while SOD2a expression increased in pre-treated eels. Our study provides insights into the tissue-specific and gender-dependent expression patterns of SODs, and how these can be affected during different maturation protocols.

Acknowledgements. The study was funded by the Spanish MICIU (EELGONIA; RTI2018-096413-B-I00). LF and MBG have PhD grants from the Generalitat Valenciana (GRISOLIAP/2020/063) and the Universitat Politècnica de València (PAID-01-20), respectively.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ANALYSIS OF YELLOWFIN TUNA *Thunnus albacares* OVARIAN MATURATION: MOLECULAR STAGING FOR THE ASSESSMENT OF STOCK REPRODUCTIVE POTENTIAL

Bir J*[1,3], Diaz de Cerio O[1], Ortiz-Zarragoitia M[1], Luque PL[2], Kukul D[2], Zudaire I[2], Cancio I[1]

[1]CBET Research Group, Dept. of Zoology & Animal Cell Biology, Centre for Experimental Marine Biology & Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza 47, 48620, Plentzia. [2]AZTI Marine Research, Basque Research and Technology Alliance (BRTA), Herrera Kaia Portualdea z/g, 20110 Pasaia. [3]Fisheries and Marine Resources Technology Discipline, School of Life Sciences, Khulna University, Khulna, 9208, Bangladesh. *Email: joyanta.bir@ehu.eus

Yellowfin tuna (YFT) is an important commercial tropical species. Improving knowledge on key biological parameters such as fecundity is essential to minimize uncertainties in stock assessment. As a batch-spawner with asynchronous ovary organization and indeterminate fecundity, it is difficult to define its gonad maturation stage necessary to calculate spawning stock biomass. Oogenesis in teleost begins with massive accumulation of 5S rRNAs and tRNAs in oocytes, both transcribed by RNA polymerase III (Pol-III) under the control of multipeptidic transcription factors (Gtf3a to c) and inhibiting-protein Maf1. Previously, we developed easily applicable molecular indices based on accumulation of Pol-III produced transcripts that differentiate female reproductive stages quantitatively. The current study measures the differential transcriptional regulation of 5S rRNA, tRNAs and associated proteins in frozen YFT ovaries at different maturation stages along the whole reproductive cycle. Ovarian total-RNA was analysed through capillary electrophoresis in an Agilent Bioanalyzer 2100 using Nano- and Small-RNA Assays. Resulting electropherograms allowed quantifying concentrations of tRNAs, 5S, 5.8S and 18S rRNA (these last two transcribed by Pol-I) and calculating tRNA/5.8S and 5S/18S rRNA indices. Fish were ranked from earliest (high index values) to latest (low values) maturation stage, perfectly correlating with the histological ranking that grouped individuals in four gametogenic stages: previtellogenic (PV), cortical alveoli, early and advanced vitellogenic (EV & AV). Transcription of Gtf3 forming polypeptides (*gtf3ab, gtf3aa, brf2, tbpl2*) and *maf1* was analysed by qPCR revealing downregulation of *gtf3aa, gtf3ab* and *maf1* towards AV stage and indicating higher Pol-III activity in PV ovary. Thus, the molecular indexing using Pol-III regulated transcripts allows accurate, cheap and unbiased ranking of YFT oogenic stages (validated through mechanistic analysis of associated genes), with applications in the assessment of the reproductive potential of stocks in tuna fisheries.

Acknowledgements. Basque Government (IT1743-22, PROBIO-00001-INP2021-33 and BAOBAB), Spanish-MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

POSTER PRESENTATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

A GONAD SPECIFIC INSULIN-LIKE PEPTIDE INVOLVED IN OVULATION AND OVIPOSITION IN RHODNIUS PROLIXUS, A VECTOR OF CHAGAS DISEASE

Lange AB*, Leyria J, Orchard I.

University of Toronto Mississauga, Department of Biology, 3359 Mississauga Rd., Mississauga, ON, L5L 1C6, Canada. *Email: angela.lange@utoronto.ca

Insulin-like peptides (ILPs) are vital hormones involved in a wide range of physiological processes in all organisms. In insects, insulin signaling has a key role in detecting and interpreting nutrient levels for egg production. Based on publicly available transcriptomes, a new ILP named gonadulin (dilp8 in *Drosophila*) has been suggested to be expressed by the gonads (hence its new name). Although the identification of gonadulin establishes its existence, its physiological relevance remains poorly understood. *Rhodnius prolixus* is an obligate hematophagous insect and a primary vector of *Trypanosoma cruzi*, the etiological agent of Chagas disease. The gonadulin transcript is highly expressed in the reproductive system, particularly in the calyx, a structure through which eggs move into the lumen of the lateral oviducts during ovulation. The putative gonadulin receptor, a member of the leucine-rich repeat-containing G protein-coupled receptor subfamily (LGR3), is most highly expressed in the central nervous system with lower levels in the reproductive tissue and other tissues. Interestingly, when the gonadulin signaling cascade is impaired using RNA interference (RNAi), eggs are retained, primarily in the ovarioles and calyx, indicating that ovulation and oviposition are inhibited. Targeting this pathway using translational research, such as symbiont mediated RNAi, could prove useful in the control of *R. prolixus* populations and mitigate the spread of Chagas disease.

Acknowledgements. This work was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grants to Angela B. Lange and Ian Orchard.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

THE EVOLUTIONARY COMPLEXITY OF THE TRANSFORMING GROWTH FACTOR-β (TGF-β) RECEPTORS

Mascoli A[1]*, Zapater C[1], Prat F[2], Gómez A[1].

[1]*Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (CSIC) 12595 Ribera de Cabanes, Castellón, Spain. [2]Instituto de Ciencias Marinas de Andalucia (CSIC), 11510 Cádiz, Spain.
*Email: alessia.mascoli@iats.cisc.es

The transforming growth factor-β superfamily (TGF-β) is a group of proteins regulating various cellular processes such as cell growth, differentiation, development, and immune responses. The signaling pathway consists of extracellular ligands, cell surface receptors, and intracellular Smad protein. Most of this knowledge comes from mammals, where several ligands have been characterized, two functional classes of serine/threonine kinase receptors, type I and type II, recognized, and some signaling pathways elucidated. In teleost reproduction several TGF- -signaling members have key roles in sex determination, sex and gonadal differentiation, germ cell proliferation, folliculogenesis, or fertility. The large number of ligands relative to the number of receptors suggests that some ligands may share the same type I or type II receptors. Given the complexity of the superfamily and the poor knowledge in fish, the purpose of the present study was to investigate the evolution of TGF-receptors across vertebrates and characterize them in European sea bass (Dicentrarchus labrax). The phylogenetic study revealed the emergence of new members in the teleost lineages through gene/genome duplication and diversification. Type I receptors formed three clades, ALK1/2, ALK3/6, ALK4/5/7, as well as the type II receptors, grouped in BMPR2/AMHR2, TGFBR2, and ACTR2/ ACTR2B. Ancestral duplications occurred at the base of vertebrate evolution, some were lost in most species but retained in teleosts. The specific teleost genome duplication generated two isoforms of alk4 (acvr1ba, acvr1bb), alk6 (bmpr1ba, bmpr1bb), bmpr2, actrii (acvr2a, acvr2aa), and actr2b (acvr2ba, acvr2bb). Type I and type II receptors were cloned in European sea bass, and functional studies of different combinations of type I/II receptors, activated by sea bass ligands, are being initiated by performing transactivation experiments in the HEK293 cell system.

Acknowledgements. Funded by Spanish MCIN/AEI/10.13039/501100011033 and by ERDF a way of making Europe (RTI2018-094667-B-C22 and PID2021-122929OB-C32). PhD contract from GV (GRISOLIAP/2020/129) supported AM.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

GONADOTROPIN PLASMID GENE THERAPY TRIGGERS SPERMATOGENESIS IN EUROPIAN EEL (*Anguilla anguilla*)

Zapater C[1]*, Morini M[2], González-López WA[2], Borges LP[2], Pérez L[2], Asturiano JF[2], Gómez A[1].

[1]*Grupo de fisiología de la Reproducción de Peces, Instituto de Acuicultura de Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellón, Spain. [2]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València, Valencia, Spain. *Email: cinta.zapater@csic.es

The severe decline in European eel populations due to overfishing and climate change raises concerns about their reproductive success. The use of species specific recombinant gonadotropins has revealed a powerful tool to control gametogenesis, but they are not sustainable for the aquaculture sector due to their high production costs. This study aimed at evaluating the potential of gene therapy in males of European eel as an alternative approach to induce spermatogenesis in captivity. Three experimental groups were set up; i) Injected with plasmid coding for an eel single-chain follicle-stimulating hormone (scFsh), ii) injected with eel scFsh and eel single-chain luteinizing hormone (scLh) plasmids and iii) the control group injected with empty plasmid. To check for injection effectiveness, all groups were injected with a small amount of sea bass scFsh, which can be monitored in plasma and has no functional effect in eel. A total of six intramuscular injections followed by electroporation were performed during the 77 days that lasted the experiment. Periodic blood samplings were performed and gonad samples were collected in the final sampling. Biometric parameters, testicular development and circulating plasma levels of sex steroid hormones were analyzed. The results show that both treatments were able to trigger spermatogenesis in male European eels, reaching spermiation in some cases. However, the combination of Fsh and Lh plasmids had a greater effect with less variability. In conclusion, compared with other type of hormonal treatments, the low cost of production and the efficiency of gonadotropin plasmid gene therapy shows the potential of this methodology to contribute to solve reproductive dysfunctions in European eel aquaculture.

Acknowledgements. This research was supported by MCIN /NextGenerationEU (PRTR-C17.I1) /Generalitat Valenciana (THINKINAZUL/2021/012; THINKINAZUL/2021/042), including the contracts of CZ and MM. WAG-L has a Margarita Salas postdoctoral contract (RD 289/2021. UAB) by the Spanish Ministry of Universities.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

IN VITRO EFFECTS OF TEMPERATURE ON STEROIDOGENIC CAPACITY OF OVARIAN FOLLICULAR CELLS IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Sarih S[1,2]*, Zapater C[1], Ibañez S[1], Gómez A[1].

[1]Fish Reproductive Physiology Group, Department of Fish Physiology and Biotechnology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castelló, Spain. [2]Aquaculture Research Group, IU-ECOAQUA, University of Las Palmas de Gran Canaria (ULPGC), Crta. Taliarte s/n, 35214 Telde, Las Palmas, Spain.
*E-mail: sarih.samira@gmail.com

In European sea bass and other fish species it is stablished that temperature may alter sex ratios by affecting estrogen production during sex differentiation. Specifically, sea bass larvae reared at high temperature develop as males by inhibition of the expression of *cyp19a1*, the gene that codes for aromatase, which is the final enzyme responsible for estrogen synthesis. However, the effect of high temperature on steroidogenesis in adult fish gonads and its underlying molecular mechanisms are less studied, although this knowledge may become relevant in the scenario of climate change. In this study, ovarian follicular cells of adult female European sea bass (*Dicentrarchus labrax*) were used as model to evaluate *in vitro* the effects of temperature on steroidogenesis in a marine teleost fish. To this aim, isolated ovarian follicular cells were grown as monolayer in multi-well plates, and incubated at two different temperatures, 15 ºC (control) and 25 ºC (high temperature). The exogenous steroid precursors cholesterol, 17α-hydroxyprogesterone or 4-androstene-3,7-dione were added to the culture medium, and after 24 and 48 hour treatments, cells and media were collected and stored for analyzing gene expression and sex steroids, respectively. According to the results, the ovarian follicular cells were steroidogenically active at both culture temperatures as observed by the secretion of testosterone and 17β-estradiol to the culture medium. Exposure to high temperature (25 ºC) had no apparent impact on secreted steroid levels. However, maintenance of ovarian follicular cells at elevated temperatures significantly reduced the expression of the gonadal aromatase gene *cyp19a1*. This study demonstrates the thermal impairment of a key steroidogenic gene at the gonadal level in the European sea bass without disturbances affecting steroid synthesis.

Acknowledgements. This research was supported by MICIN /NextGenerationEU (PRTR-C17.I1) /Generalitat Valenciana (THINKINAZUL/2021/042). S.Sarih was supported by a research contract from Margarita Salas program from ULPGC.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TRANSIENT RECEPTOR POTENTIAL VANILLOID (TRPV) ION CHANNEL IN THE EUROPEAN EEL UNDER DIFFERENT CONDITIONS

Ferrão L[1]*, Cottin D[2], Asturiano JF[1], Dufour S[2], Morini M[1].

[1]Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain. [2]Laboratory Biology of Aquatic Organisms and Ecosystems (BOREA), National Museum of Natural History (MNHN), CNRS, IRD, Sorbonne University, Paris, France. *E-mail: mlpirfer@posgrado.upv.es

Global warming threatens wildlife and poikilotherm organisms, being this last particularly vulnerable to temperature changes. TRP channels constitute a superfamily of receptors involved in sensorial functions. Among these, the transient receptor potential vanilloid (TRPV) are known to play roles in thermosensing. Previously, we identified three TRPV genes (TRPV1, 4, and 6) in the European eel but their physiological functions remain unknown. Considering the different environmental conditions encountered during eel migration it could be interesting to study their expression. First, we described TRPV genes expression in the European eel in both genders. Tissue screening was studied in female and male eels and showed higher TRPV expression in female than in male tissues. TRPV4 showed a prominent expression in the brain-pituitary-gonad axis tissues of females and peripheral tissues of males. TRPV6 displayed a high expression in the ovaries of females, but low levels in gills, fat, and brain. In males, TRPV6 was detected in the testis, liver, and brain. Then, we described TRPV expression in the pituitary, gills, skin, and testis at different salinities (freshwater vs seawater) and temperatures (10 vs 20 °C), that simulate eel migration conditions. Salinity and/or temperature affected the expression of TRPV1, 4 and 6 in the pituitary and skin, but only TRPV4 and 6 in the gills. The highest TRPV expression has been detected in the testis, but the response to salinity and temperature varied among individuals. Our study revealed the different TRPVs expression patterns in the European eel and suggested the implication of the three eel TRPVs in the sensitivity to temperature and salinity changes.

Acknowledgements. Study supported by the Spanish MICIU (EELGONIA; RTI2018-096413-B-I00), and MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (THINKINAZUL/2021/012) to SEASPERM. LF and MBG have PhD grants from the Generalitat Valenciana (GRISOLIAP/2020/063) and the Universitat Politècnica de València (PAID-01-20), respectively.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TISSUE SPECIFIC TRANSCRIPTIONAL PROFILES OF AUTOPHAGOCYTOSIS AND LYSOSOMAL GENES: TOWARDS UNDERSTANDING ATRESIA IN FISH OOCYTES

Atzori C*, Bir J, Diaz de Cerio O, Cancio I.

CBET Research Group, Dept. of Zoology & Animal Cell Biology, Centre for Experimental Marine Biology & Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza 47, 48620, Plentzia.
*Email: catzori001@ikasle.ehu.eus

The fate of oocytes during differentiation depends on interconnected genetic, metabolic, physiological and environmental factors. The mechanistic target of rapamycin (mTOR) could be the integrator of the nutritional, hormonal and stress status of the individual leading to oocyte growth and proliferation or otherwise to oocyte follicular atresia and death. Indeed, mTOR controls ribosomal biogenesis pathways during growth that would allow protein synthesis in the early embryo or alternatively trigger autophagocytosis for resorption and recovery of energy expenditure. Under starvation or others kind of stress, mTOR is inactivated leading to activation of ULK1/2 kinase and initiation of autophagocytosis. mTOR also upregulates TFEB, master transcriptional regulator of lysosomal biogenesis genes such as the lysosomal membrane protein coding *lamp2*. Firstly, *Chelon labrosus* ESTs coding for *ulk1, tfeb* and *lamp2* were obtained through gonadal RNA-Seq analysis, and then, the transcription of the three genes was studied through qPCR in different tissues, including ovaries, testes, and intersex testis of xenoestrogen exposed mullets. Similar analyses were conducted also on zebrafish (*Danio rerio*) tissues. Transcription of the three genes was ubiquitous in both species with a higher transcription for the ovaries in the case of *Chelon*. In mullets, whose ovaries were at previtellogenesis stage, transcription levels were significantly higher in ovaries than in testes, with intersex testes showing a transcription profile in between both tissues. A histochemical technique using β-glucuronidase as marker enzyme was also validated, granting the microscopic visualization of large lysosomes in the cytosol of mullet vitellogenic oocytes. The generated qPCR and histochemical methods will allow the study of autophagocytosis and lysosomal activity in fish oocytes under atresia triggered by nutritional or chemical stress.

Acknowledgements. Basque Government (IT1743-22) and Spanish MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

mTOR GENE SEQUENCING IN *Chelon labrosus* AND GENE TRANSCRIPTION LEVELS IN FISH TISSUES

Urkixo E, Atzori C, Bir J*, Diaz de Cerio O, Cancio I.

CBET Research Group, Dept. of Zoology & Animal Cell Biology, Centre for Experimental Marine Biology & Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza 47, 48620, Plentzia.
*Email: joyanta.bir@ehu.eus

During fish oogenesis, oocytes must incorporate molecules such as ribosomal RNAs to allow growth of the future embryo. 5S rRNA and tRNAs can make up 75% of the total RNA in fish ovary, to ensure the production of ribosomes and thus protein synthesis that will be necessary in the newly formed embryo. Polymerase III is the responsible of 5S rRNA and tRNA synthesis, and its activity depends on mTOR. mTOR controls cell metabolism, growth, survival and autophagy depending on nutrient availability, hormonal and growth factor signalling and stress condition. Under optimal conditions, mTOR ensures cell growth and proliferation while under stress it triggers autophagy. Considering all this, it is important to analyze the role of mTOR in the physiology of fish gonads. 77% of the *Chelon labrosus mtor* CDS was obtained through the alignment of three ESTs identified from a RNA-Seq of mullet gonads, sequence information obtained from the NCBI SRA repository and PCR and sequencing analysis of mullet cDNA using specifically designed primers. Comparing the sequence obtained with the orthologous *Mugil cephalus mtor* high sequence homology was inferred, and the presence of conserved protein regions typical of a serine/threonine kinase identified. The transcription of *mtor* in *C. labrosus* and *Danio rerio* tissues was ubiquitous. In mullets, transcription levels were significantly lower in the ovary than in testis or liver. Interestingly, intersex testes of xenoestrogen exposed mullets showed transcription patterns more similar to the ovary than to the normal testis. In zebrafish, both the testis but mainly the ovary showed lower transcription levels than the other tissues. The results show ubiquitous transcription of *mtor* with a reduction of transcription in tissues presenting oocytes (ovary or intersex testis). Future studies should analyze the transcription of the gene in the different stages of ovarian development under different feeding regimes and stress conditions.

Acknowledgements. Basque Government (IT1743-22) and Spanish MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

DESCRIPTION OF MANILA CLAM POPULATION IN ARCACHON BAY (FRENCH ATLANTIC COAST) BASED ON BIOLOGICAL FEATURES AND LOCAL ENVIRONMENTAL PARAMETERS

Briaudeau T[1]*, Sanchez F[2], Bru N[3], de Montaudouin X[4], Benito D[1], Ruiz P[1], Izagirre U[1], Lissardy M[2], Caill-Milly N[2].

[1]*CBET Research Group, Dept of Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Basque Country, Spain. [2]Ifremer, LITTORAL, F-64600 Anglet, France. [3]University of Pau & Pays Adour / E2S UPPA, Laboratory of Mathematics and its Applications of PAU - MIRA, UMR 5142 CNRS, F-64600 Anglet, France. [4]University of Bordeaux, UMR 5805 EPOC CNRS, Marine Station of Arcachon, F-33120 Arcachon, France.

*Email: tifanie.briaudeau@ehu.eus

Introduced in France in the 70s, the Manila clam (*Ruditapes philippinarum*) is a neonaturalized species that became economically important in Arcachon Bay (SW of France). Research has been carried out since the 2000s to survey the population dynamics. The present study reviews environmental and biological data collected within that period to identify confounding factors explaining variability within the population. Studied biological characteristics include growth, reproduction and mortality data recorded for Manila clams from four sampling sites across the lagoon. Clams from different sites vary in asymptotic shell length and size frequency. They are characterized by different morphologies with more globular and heavier individuals in Andernos, elongated animals in Lanton and lighter individuals in Gujan. The reproduction of the Manila clam displays some temporal and spatial variability throughout the bay with spawning period differing by a month and number of spawning events varying according to sites and years. The lengths at first sexual maturity (SL_{50}) also differ moderately between sites with highest SL_{50} in Andernos and lowest and more variable SL_{50} in Île aux Oiseaux. Among studied environmental parameters, temperature and trophic sources are two main drivers influencing clam growth and reproduction. As Arcachon Bay is a transitional basin, these parameters vary throughout the lagoon based on the Western oceanic and the Eastern fluvial influences. Biological elements such as the presence of pathogens and development of diseases can also affect the species evolution. In this case, perkinsosis is known to affect the Manila clam growth and the Brown Muscle Disease inhibits burrowing behavior, increasing mortality.

Acknowledgements. Studies were supported from National, Regional, European funds (for details, refer to the published articles on these topics) and relied on a partnership with Ifremer, fishing professional organizations and academic institutes. TB profited from a European Union-Next Generation postdoctoral fellowship.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

LIVER AND PLASMA FATTY ACID PROFILES IN FEMALE BROWN TROUT ALONG DISTINCT REPRODUCTIVE STAGES

Madureira TV[1,2]*, Santos D[1,2], Simões T[3], Lemos MFL[3], Rocha E[1,2].

[1]Team of Animal Morphology and Toxicology, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto (U.Porto), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. [2]Laboratory of Histology and Embryology, Department of Microscopy, ICBAS — School of Medicine and Biomedical Sciences, University of Porto (U.Porto), Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. [3]MARE-Marine and Environmental Sciences Centre & ARNET — Aquatic Research Network Associated Laboratory, ESTM, Polytechnic of Leiria, 2520-641 Peniche, Portugal. *Email: tvmadureira@icbas.up.pt.

Fatty acid profiles in fish are recognizable indicators of distinct environmental conditions, such as contamination, temperature, salinity but also physiological changes, including those related to the reproductive cycle. Within this context, this study proposes to evaluate the variation of fatty acids in female adult brown trout in the liver and plasma, with the main aims of: I) trace for the first time the females' profile of fatty acids in this species, in both types of biological sample and II) carry out a screening of the most representative fatty acids in the different reproductive stages, namely spawning capable (December), regressing (March), regenerating (July) and developing (November).

For fatty acid classes, distinct patterns were noted along the four reproductive stages. In the liver, high monounsaturated fatty acids (MUFA) levels and low polyunsaturated fatty acids (PUFA) contents were obtained at regressing and regenerating stages, while an inverse profile was observed during spawning capable and developing stages. For plasma samples, PUFA were mainly responsible for distinguishing reproductive stages, with higher levels being obtained at spawning and regressing stages.

The most representative fatty acids were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n−9), arachidonic acid (20:4 n−6), eicosapentaenoic acid (EPA, 20:5 n−3) and docosahexaenoic acid (DHA, 22:6 n−3). Plasma samples allowed the distinction of the four reproductive stages based on the most representative fatty acids. For this reason, those FA may be regarded as biomarkers of female gonad status in brown trout.

Acknowledgements. Strategic funding to CIIMAR (UIDB/04423/2020 and UIDP/04423/2020), to MARE (UID/MAR/04292/2020), and to the Associate Laboratory ARNET (LA/P/0069/2020), through national funds provided by FCT — Fundação para a Ciência e a Tecnologia. T. Simões was supported by an FCT researcher contract (2021.02559.CEECIND). We also thank the ICNF — Instituto da Conservação da Natureza e Florestas and the Posto Aquícola do Torno facilities.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

MOLECULAR CHARACTERIZATION AND GONAD EXPRESSION PATTERN OF DEAD-END (*dnd*) GENE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Djellata A[1,2]*, Zapater C[1], Ibañez S[1], Gómez A[1].

[1]Fish Reproductive Physiology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castelló, Spain. [2]Aquaculture Research Group, IU-ECOAQUA, University of Las Palmas de Gran Canaria (ULPGC), Telde, Las Palmas, Spain.* E-mail: djellata_adnane@hotmail.fr

Dead end (*dnd*) is a germ cell-specific RNA maternally provided within the germ plasm. It encodes an RNA-binding protein that plays an indispensable role in the development and migration of primordial germ cells, the cells responsible for transferring genetic information to the offspring. This gene has been characterized in many vertebrate lineages and in the last years has been used as target to induce sterility in a few fish species. However, there is a lack of information about this gene and its expression pattern in European sea bass (*Dicentrarchus labrax*). In this study, and departing from different genome predictions, we have determined the whole coding sequence of sea bass *dead-end* (*sb-dnd*) cDNA, set up a specific qPCR assay and analyzed its expression levels in different sea bass tissue samples.

Measurement of *sb-dnd* mRNA levels in different adult tissues from male and female sea bass revealed its gonad-specific expression. In gonads from adult fish *sb-dnd* transcripts were detected both in the testes and ovaries during a whole annual reproductive cycle, with higher expression in ovary than in testis. In ovaries, expression of *sb-dnd* was high and stable during previtelogenic stages, decreasing as vitelogenesis and maturation progressed, and rising sharply in post-spawning, coinciding with the proliferation of oogonia and generation of new oocytes. In testis, highest expression of *sb-dnd* was found in spermatogonia-containing and premeiotic stages and decreased during spermatogenesis. Expression of this gene was also analyzed in embryos, newly hatched larvae and gonads of prepubertal animals. All together these results offer insight in the potential use of *dnd* as target to induce sterility in European sea bass.

Acknowledgements. This research was supported Spanish MCIN/AEI/10.13039/501100011033 and by ERDF a way of making Europe (PID2021-122929OB-C32). A.Djellata was supported by a research grant from Margarita Salas program from ULPGC.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SEX STEROIDS AS ENDOCRINE AND PARACRINE REGULATORS OF GAMETOGENESIS IN FEMALE EUROPEAN SEA BASS (*Dicentrarchus labrax*).

Zapater C[1], Bort M[1], Rocha A[1,2], Ibañez S[1], Gómez A[1]*.

[1]Grupo de Fisiología de la Reproducción de Peces, Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castelló, Spain. [2]Present address: Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Matosinhos, Portugal. *Email: a.gomez@csic.es

The European sea bass is a teleost of great economic importance in the Mediterranean aquaculture. During the last years we have learned about the role of endocrine signals in puberty and gonadal development, but mostly related to gonadotropin action in males. In the present study we aimed to investigate the role of sex steroids as endocrine or local factors in oogenesis. For this purpose, the levels of each of the main sex steroids (testosterone, 17β-estradiol, 11-ketotestosterone and 17α, 20β-dihydroxy-4-pregnen-3-one) were quantified in ovary and plasma of adult females during a whole reproductive cycle. In addition, we isolated the cDNAs of new duplicated counterparts coding for steroidogenenic acute regulatory protein (StAR) and 3β-hydroxysteroid dehydrogenase (3β-HSD), two important steroidogenic enzymes, and studied the expression of these isolated genes and of their duplicates that were already available for sea bass. The results showed that 17β-estradiol accumulates in the ovary during vitellogenesis, indicating a local role in addition to its known endocrine action inducing vitellogenin synthesis in the liver. Testosterone and 11-ketotestosterone gain importance in the ovary at the end of vitellogenesis, and the shift to progestogen production occurs already at the posvitellogenic stage. In relation to the steroidogenic enzymes, the StAR (*star* and *mstar*) and 3β-HSD (*3βhsd* and *3βhsd7*) coding genes showed different tissue expression profiles between the duplicates, as well as different trends in ovarian expression during the annual cycle. The new *mstar* gene shows highest expression during maturation and ovulation, which would be related to progestogen production, similar to *hsd3b* expression, while *hsd3β7* expression may be more related to 17β-estradiol production. In conclusion, these findings contribute to our understanding on the role of sex steroids as endocrine and local regulators during gametogenesis in female European sea bass.

Acknowledgements. Funded by Spanish MCIN/AEI/10.13039/501100011033/ and by ERDF a way of making Europe (AGL2015-67477-C2-1-R, PID2021-122929OB-C32).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

MOLECULAR MARKERS OF FOLLICULAR ATRESIA IN THICKLIP GREY MULLETS (*CHELON LABROSUS*) INHABITING IN AN ESTUARY (GERNIKA) WITH HIGH BURDENS OF XENOESTROGENS

Nzioka A*, Valencia A, Rojo-Bartolomé I, Lekube X, Ortiz-Zarragoitia M, Diaz de Cerio O, Cancio I.

CBET Research Group, Dept. Zoology & Animal Cell Biology; Faculty of Science & Technology and Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza Hiribidea 47, 48620 Plentzia, Basque Country, Spain. *Email: anthony.nzioka@ehu.eus

Follicular atresia is a degenerative process involving apoptosis and autophagy related mechanisms that is essential for the maintenance of ovarian homeostasis in teleost fish. This common phenomenon during oogenesis is responsible for the fish fitness and is associated with environmental factors, changes in hormonal levels or the end of spawning season. With xenoestrogenic effects having been reported in populations of thicklip grey mullets *Chelon labrosus* from contaminated estuaries in the Bay of Biscay, this study investigated the morphological changes and expression levels of various genes and proteins involved in apoptosis and autophagy during follicular atresia through qPCR and immunoblotting. 31 female mullets sampled downstream of the wastewater treatment plant of Gernika during summer from 2014 to 2019 were analysed histologically to identify the prevalence of atresia in this population exposed to xenoestrogens. The prevalence of intersex in males captured together with these males was between 10% and 30%. Ovaries were always in previtellogenesis as it is normal in June-July. Histopathological analysis revealed a high prevalence of atresia (up to 100% one of the years) in previtellogenic ovarian follicles, with high number of basophilic nuclei and shrunk oocytes detached from neighbouring follicles and connective tissue. Transcripts of apoptosis and autophagy-related genes *p53, mdm2, rpl5, caspase-3* and *beclin-1* revealed no significant differences in previtellogenic ovaries with or without atretic follicles. Immunoblot analysis revealed significant differences in protein content of both p53 and RPL11 between ovaries with and without atretic follicles. The results show that not only does follicular atresia affect previtellogenic ovarian follicles in fish environmentally exposed to xenoestrogens, but that also p53 has a key role to play in apoptosis-mediated atresia in teleosts and its activation is most probably regulated post-transcriptionally. Whether these effects have implications on the reproductive capacity of mullet populations remains unclear.

Acknowledgements. This work was funded by the Spanish Ministry of Science Innovation and Universities and EU-FEDER/ERDF (ATREoVO AGL2015-63936-R and BORN2bEGG PGC2018-101442-B-I00), the Basque Government (Grants to consolidated research groups IT1302-19 and IT1743-22). AN is a recipient of a pre-doctoral grant from the UPV/EHU.

SESSION 2.
NEUROENDOCRINOLOGY
AND
RHYTHMS

PLENARY LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CIRCADIAN ROLES OF THE MELANOCORTIN SYSTEM

Godino-Gimeno A[1], Leal E[1], Tormos-Ribes E[1], Chivite M[2], Míguez JM[2], Rotllant J[3], Cerdá-Reverter JM[1]*.

[1]*Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, 12595 Castellon, SPAIN. [2]Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, 36310 Vigo, Spain. [3]Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas (IIM-CSIC), 36208 Vigo, Spain.
*Email: jm.cerda.reverter@iats.csic.es

Melanocortins are peptides derived from the post-transcriptional processing of proopiomelanocortin precursor (POMC), which encodes several melanocyte-stimulating hormones (MSHs) and adrenocorticotropic hormone (ACTH). Five different receptors (MC1R-MC5R) with discrete functional domains and binding profiles mediate melanocortin signalling. Atypically, the melanocortin system is also regulated by an endogenous antagonist such as agouti-signalling protein (A SIP) and agouti-related protein (AGRP). The melanocortin system is essential for the regulation of food intake, stress and pigmentation in vertebrates, but only a few studies have focused on its role in behaviour. Here, we review our recent experiments focusing on the involvement of the melanocortin system in the regulation of circadian rhythms using a zebrafish strain overexpressing asip1 (asip1-Tg). We compared the daily activity rhythms of asip1-Tg and WT strains and found that the circadian activity pattern was completely disrupted in the transgenic animals, which perfectly matched the reduced nocturnal serotonin and melatonin levels in the asip1-Tg animals. The expression of clock genes was also disrupted and the rhythmicity of per1a and clock1a genes was masked by overexpressing asip1. A direct role of ASIP1 in melatonin secretion was confirmed using in vitro pineal culture, which showed a dose-related inhibitory effect on melatonin secretion. Data support that the inhibition of melanocortin system by endogenous antagonists disrupts the central circadian clock masking melatonin secretion and thus the daily activity patterns.

Acknowledgements. This study was financed by funds from MCIN/AEI/ 10.13039/501100011033 (PID2022-136288OB-C33) and, as appropriate, by “ERDF A way of making Europe”, by the “European Union” or by the“European Union Next Generation EU/PRTR”.

ORAL COMMUNICATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

FOOD ANTICIPATORY ACTIVITY IS AN ANXIOUS CONDITION IN *Carassius auratus* THAT IS MEDIATED BY GHRELIN

Herrera-Castillo L*, Chen P, Saiz N, Delgado MJ, De Pedro N, Isorna E.

Fish Neuroendocrinology Research Group, Department of Genetics, Physiology and Microbiology, Faculty of Biological Sciences, Complutense University of Madrid, Spain. *Email: lisbethh@ucm.es

Food anticipatory activity (FAA) is an increase in locomotor activity that occurs before the expected mealtime. It is related not only with a food-seeking mediated by hunger, but also with expectations of food reward. We hypothesize that FAA could imply a state of anxiety related with this search and desire for food. The mechanisms that drive FAA are still unknown, but it has been suggested that ghrelin, a potent orexigenic gut hormone, could be involved on its onset. The aim of this study was to determine whether the FAA in fish could be considered as an anxious state, and the possible involvement of ghrelin. Anxiety was evaluated in goldfish using open field and black-white behavioral tests. These tests were carried out at three different daytimes: (1) after 24 h fasting (FAA time); (2) at 3 h postprandial; and (3) after 30 h fasting (6 h fasted after FAA period). Goldfish showed higher thigmotactic and scototactic behavior during the FAA than after (in both, fed and unfed animals), suggesting that FAA is an anxiogenic state independently of fasting. To determine if ghrelin mediates such anxiogenic behavior unfed fish were injected with ghrelin antagonists (D-Lys and JMV2959, 100 pg/g body weight, bw); and fed goldfish were treated with ghrelin alone (10 pg/g bw) or ghrelin with its antagonist (D-Lys). Both antagonists decreased thigmotaxis and scototaxis responses, as well as the latency to the open and white areas. Ghrelin, like the FAA, induced a state of anxiety, ghrelin-injected fish showing less entries into the open field and a higher latency to this area, effects that were reversed by its antagonist. Overall, the results show that ghrelin has an anxiogenic effect in goldfish, and this effect may be involved in the food seeking that occurs during the FAA.

Acknowledgements. Supported by the Spanish MICIU (PID2019-103969RB-C32). L.H.C and N.S. are predoctoral fellow from UCM (CT42/18-CT43-18 and CT58/21-CT59/21).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CHARACTERIZING THE PEPTIDERGIC [ARG8]VASOTOCIN SYSTEM IN THE SEA LAMPREY (*PETROMYZON MARINUS*)

Barany A[1,2,3]*, Shaughnessy CA[4], Mancera JM[1]*, Regish A[3], McCormick SD[2], Dores RM[4].

[1]Universidad de Cádiz, Puerto Real 11519, España. [2]University of Massachusetts, Amherst, MA 01003, USA. [3]U.S. Geological Survey, Conte Anadromous Fish Research Laboratory, Turners Falls, MA 01376, USA. [4]University of Denver, Denver, CO 80210, USA. *Emails: andre.barany@uca.es; juanmiguel.mancera@uca.es

Sea lamprey (P*etromyzon marinus*) is a jawless vertebrate that undergoes a larvae-to-juvenile metamorphosis, which includes the development of tolerance to seawater (SW). While the fundamental mechanisms of osmoregulation in lampreys seem to resemble those of teleosts, the role of neurohypophysial hormones in controlling osmoregulation in freshwater (FW) or SW is poorly understood. The precursor peptide gene arginine vasotocin (VT) existed as a single gene before vertebrates originated. Today, multiple gene orthologs (AVP, oxytocin -OT-, IT, and MT) are present across various vertebrate species. This study aimed to investigate physiological and pharmacological functions of VT and their receptors in sea lamprey through a series of *ex vivo*, *in vitro,* and *in vivo* approaches to understand their role in supporting osmoregulation. We examined transcriptional expression of the neurohypophysial prohormone (*vt*) and receptors (*vtr1ra, vtr1rb, vtr1rc, vtr2ra, vtr2rb*) in tissues from sea lamprey: i) acclimated to FW and SW, as well as pre-and post- metamorphosis; ii) juveniles submitted to stress by air exposure (1 min) plus crowding (10 min) and iii) fed juveniles. Additionally, we studied VT effects on *ex vivo* intestinal water absorption (from FW and SW acclimated specimens) and *in vivo* systemic osmoregulation in FW acclimated juvenile. Furthermore, we conducted *in vitro* experiments to investigate the stimulation of lamprey V1- and V2-type receptors using VT and OT as well as their potential G protein-coupled receptor associations. This research represents the first comprehensive analysis of the role(s) of VT at different levels, ranging from molecular function to physiological action, in the sea lamprey.

Acknowledgments. AB is a Margarita Salas Postdoctoral Fellow at the University of Massachusetts (Amherst, USA), supported by Universidad de Cádiz (Spain) and by The Company of Biologists Limited in the University of Denver. National Science Foundation Postdoctoral Fellowship (DBI-2109626) to CAS and PID2020-117557RB-C22 grant to J.M.M. (AEI-MINECO; Spain).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

LIGHT PHOTOPERIOD AND SPECTRUM MODULATE DAILY RHYTHMS OF PROLIFERATION, APOPTOSIS, DNA REPAIR AND OXIDATIVE STRESS MARKERS IN FISH EMBRYONIC STEM CELLS

Vergès-Castillo A[1]*, Herrera-Pérez P[1], Pendón C[2], Martín-Robles AJ[2], Muñoz-Cueto JA[1].

[1]*Department of Biology, Faculty of Marine and Environmental Sciences, INMAR, and [2] Department of Biomedicine, Biotechnology and Public Health, Faculty of Sciences, University of Cádiz, Puerto Real, Spain.
*Email: alba.verges@uca.es

In fish, the light-dark cycle represents the most powerful abiotic factor for the initiation and synchronization of different rhythmic processes, providing not only daily but also seasonal temporal information to the organisms. In aquatic environments, water acts as a chromatic filter that modulates light intensity and wavelength penetration into the water column, with blue and green wavelengths penetrating more deeply, while UV/violet and red wavelengths are absorbed more quickly and only reach shallower depths. In fish, light is absorbed by photopigments (opsins) of photoreceptor cells present in the fish pineal gland and retina, and transduced into neural (neurotransmitters) and neurohormonal (melatonin) signals that synchronize biological rhythms in central and peripheral tissues and cells. But in some fish species, such as the zebrafish, light can acts directly on peripheral organs and cells, which exhibit a wide variety of non-visual opsins. In previous works, we have established and characterized single cell-derived embryonic stem cell lines from the gilthead seabream, *Sparus aurata*, and revealed that they exhibited a rhythmic molecular clock machinery, which is entrained by the light photoperiod. In the present work, we analysed the *in vitro* effects of light photoperiod and spectrum on daily gene expression of different markers of cell proliferation (*pcna*), apoptosis (*bcl2*, *bax*), DNA repair (*cry5*) and oxidative stress (*hsp70*, *prx2*) by using RT-qPCR. Our results revealed that fish embryonic stem cells are sensitive to light photoperiod and spectrum, with blue lights providing the best results for inducing gene expression and for the entraining of robust daily rhythms of cell proliferation, apoptosis, DNA repair and oxidative stress markers. These results should be taken into account when performing *in vitro* studies with cultured fish cells.

Acknowledgements. This research was supported by the Spanish Ministry of Science and Innovation under grant No PID2021-123640OB-C22 (BBLUEAQUA) to JAM-C and AJM-R.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

DAILY RHYTHMS OF THERMAL PREFERENCE IN DIURNAL AND NOCTURNAL FISH

de Alba G[1], Pintos S[1], Conti F[1], Godoy L[1], Santos S[2], Szewczyk TM[2,3], Geralda Campos F[4], Mackenzie SA[2], López-Olmeda[1] JF, Sánchez-Vázquez[1] FJ, Rey-Planellas S[2], Vera LM[1], Sánchez J[1]*.

[1]*Department of Physiology, Faculty of Biology, Regional Campus of International Excellence ''Campus Mare Nostrum'', University of Murcia, 30100 Murcia, Spain. [2]Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK. [3]The Scottish Association for Marine Science, SAMS, Dunbeg, Oban, Argyll, PA37 1QA, UK. [4]Department of Anima Science, Federal University of Viçosa, MG, 36570-900, Brazil. *Email: javisan@um.es

Thermal preference allows fish to choose the time of day with the best thermal conditions to develop their biological processes. The aim of this study was to determine the influence of the daily activity on the daily rhythms of thermal preference of diurnal zebrafish (*Danio rerio*) and Nile tilapia (*Oreocrhomis niloticus*) and nocturnal tench (*Tinca tinca*) under a 12h:12h Light:Dark cycle. For this purpose, using a multichamber tank with different fish specific thermal gradient (from 24 ºC to 32 ºC, 26 ºC to 34 ºC and 18 ºC to 26 ºC for zebrafish, Nile tilapia and tench, respectively), the fish were allowed to freely choose the daily preferred temperature. Regardless the daily activity of fish, all fish species displayed consistent daily rhythms of thermal preference with higher temperatures being selected during the second half of the light phase and lower temperatures during the night phase, with acrophases at Zeitgeber Time (ZT) 5.37h (zebrafish), 6.55h (tench) and 12.5h (tilapia). Interestingly, when moved to the experimental tank, only tilapia displayed higher temperature preference. Our findings suggest that daily rhythm in thermal preference is species-specific and it could be related to daily changes of environmental temperature that fish experiences in the wild (warming up during day and cooling down at night). These results highlight the importance of integrating both light driven-daily rhythm and thermal choice to refine our understanding of thermal biology of fish.

Acknowledgements. This research was funded by Project "BLUESOLE" (AGL2017-82582-C3-3-R) and "BBLUEAQUA" (PID2021-123640OB-C21) granted by the Spanish Ministry of Economic Affairs and Competitiveness (MINECO) to FJSV and LMV; Grant RYC-2017-21835 ("Ramón y Cajal" fellowship) awarded to LMV by the Spanish MINECO/AEI/10.13039/501100011033 cofunded by "ESF Investing in your future" TS and SRP was funded by the BBSRC OffAqua project (Evaluating the Environmental Conditions Required for the Development of Offshore Aquaculture) – BB/S004246/1.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ANTIDEPRESSANTS – THE NEW ENDOCRINE DISRUPTORS?

Knigge T[1], Monsinjon T[1], Zapater C[2], Gomez A[2], Power D[3], Pinto P[3].

[1]Normandie Univ, Unilehavre, FR CNRS 3730 Sciences Appliquées à L'Environnement, UMR-I02, Environmental Stress and Biomonitoring of Aquatic Environments, University of Le Havre Normandy, Le Havre, France. [2]Instituto de Acuicultura Torre la Sal, Ribera de Cabanes, 12595 Castellón, Spain. [3]CCMAR-Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

*Email: thomas.knigge@univ-lehavre.fr

Selective serotonin and noradrenaline reuptake inhibitors (SSRIs/SNRIs) figure amongst the high-volume pharmaceuticals. Due to an increasing variety of antidepressant medication and prescription rates, SSRIs/SNRIs are found in surface waters and accumulate to concentrations in the µg to mg·L^{-1} range. Although not considered canonical endocrine disrupting chemicals (EDCs), a number of studies have reported effects of SSRIs/SNRIs on steroidogenesis and steroid hormone signalling in vertebrates. Using a reporter gene assay with the three *Dicentrarchus labrax* estrogen receptors (Esr1, Esr2a, Esr2b) and the two membrane oestrogen receptors (Gpera and Gperb), we did not observe any oestrogenic activity of fluoxetine with any Esr. In the presence of 17β-oestradiol (E2), however, fluoxetine displayed antiestrogenic activity through Esr1 and Esr2b. On the contrary, fluoxetine transactivated the two Gpers without any interaction with E2. These results confirm the potentially endocrine disrupting potential of antidepressants with respect to sex steroid signalling in vertebrates.
It is widely accepted that canonical EDCs do not interfere with the invertebrates' endocrine systems, either due to lack of steroid hormones, or due to fundamental differences in receptor types and receptor activation. Given that invertebrate endocrinology is mainly neuroendocrine in nature, invertebrate endocrine systems would appear to be natural targets for antidepressants. Indeed, we could show that fluoxetine stimulated the release of crustacean hyperglycaemic hormone leading to the mobilisation of glucose and increased locomotor and burying activity in the European shore crab *Carcinus maenas*. Furthermore, fluoxetine stimulated the release of moult inhibiting hormone resulting in significantly lowered 20-hydroxyecdysone levels indicative of endocrine disruption of moulting. Exposure of juvenile Shore crabs to a mixture of fluoxetine and venlafaxine significantly reduced the animal's colour change capacity, putatively by stimulating pigment dispersing hormone, thereby compromising camouflage. The results provide indirect evidence for antidepressant derived modulation of several neuropeptide hormones by altering neurotransmitter levels.

Acknowledgements. This research was supported by funding via the AQUAEXEL programme (AE050029), the French ANR ETaT (ANR-15-CE32-0014), the French-English Interreg IVa PeReNE (4230), the Spanish MCIN/AEI/10.13039/501100011033 and by ERDF a way of making Europe (AGL2015-67477-C2-1-R, PID2021-122929OB-C32), RTI2018-094667-B-C22), the Portuguese Foundation for Science and Technology (FCT) through projects UIDB/04326/2020 and PTDC/AAG-GLO/4003/2012 as well as a researcher contract to PP under "Norma Transitória"-DL57/2016/CP1361/CT0015.

POSTER PRESENTATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SEROTONIN TARGETS THE CIRCADIAN CLOCK IN RAINBOW TROUT. PRELIMINARY RESULTS.

Alborja-Valado M*, Ramírez-Rodriguez CA, Chivite M, López-Patiño MA, Míguez JM.

Centro de Investigación Marina, Laboratorio de Fisiología Animal, Departamento de Biología Funcional y Ciencias de la Salud, Facultad de Biología, Universidad de Vigo, Vigo, España. *Email: maria.alborja@uvigo.es

The monoamine serotonin is widely used as a neurotransmitter in vertebrates. In mammals, it is present in the central nervous system and in peripheral tissues, particularly in the intestine where it reaches the highest concentrations. At the brain level, serotonergic neurons project from the mesencephalic raphe nuclei to large areas of the diencephalon and telencephalon, including the supraschiasmatic nucleus of the hypothalamus, which houses the circadian master clock. There is multiple evidence that serotonergic signaling evokes changes in the phase of circadian rhythms and modulates their adjustment by environmental photoperiod and internal signals. Moreover, at the intestinal level, serotonin has also been implicated in circadian regulation acting through specific receptors. In fish, the functional role of serotonin in circadian regulation has not yet been studied. In the present study, rainbow trout were injected (i.p.) with saline, serotonin hydrochloride and d-fenfluramine, the latter a drug that increases the vesicular release of serotonin and its synaptic concentration. The injected fish were then sampled every four hours throughout a complete daily cycle. Brain and intestinal tissues were dissected to analyze the expression (q-PCR) of several circadian clock loop genes (clock1a, bmal1, per1, cry2 and rev-erbβ-like), as well as changes in serotonin concentrations (HPLC). The results showed that while d-fenfluramine altered endogenous serotonin levels at the brain and gut level, serotonin treatment only did so at the peripheral level. Consequently, serotonin treatment altered the amplitude (clock1a, bmal1) and the phase (per1, cry2) of rhythms in the gut, but not in the brain. On the other hand, d-fenfluramine modified the amplitude and/or phase of clock gene expression rhythms in both brain and gut, with effects that were sometimes opposite to those of serotonin. The results point to a role for serotonin in circadian regulation in teleost fish, acting on molecular oscillators both centrally and in the gastrointestinal tract.

Acknowledgements. Funded by the Spanish Agencia Estatal de Investigación and European Fund of Regional Development (PID2019-103969RB-C31) and Xunta de Galicia (Consolidación e estructuración de unidades de investigación competitivas do SUG, ED431B 2019/37) to JLS.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

THE GASTROINTESTINAL CLOCK IN RAINBOW TROUT. INFLUENCE ON GASTROINTESTINAL HORMONES.

Alborja-Valado M*, Chivite M, Míguez JM, López-Patiño MA.

Centro de Investigación Marina, Laboratorio de Fisiología Animal, Departamento de Biología Funcional y Ciencias de la Salud, Facultad de Biología, Universidad de Vigo, Vigo, España. *Email: maria.alborja@uvigo.es

Feeding is a priority in aquaculture. That makes necessary to dig into the mechanisms (homeostatic, hedonic and circadian) that affect feeding behavior. Regarding circadian mechanisms in the teleost group, multiple oscillators locate in different central and peripheral tissues and interact with each other. Oscillators synchronize to different internal and external cues, such as the light-dark cycle and food availability. The gastrointestinal tract (GIT) is a key component for food digestion and nutrient absorption. Its regulation involves multiple neuroendocrine processes. With the arrival of food to the GIT, digestive activity modulators [ghrelin, cholecystokinin (CCK) and glucagon-like peptide-1 (GLP1) among others] are released, with the digestive activity occurring on a daily rhythmic basis, thus indicating that a local circadian oscillator may control GIT activity. However, very little information exists on how these processes occur in the gut of fish. Therefore, we aimed to elucidate in rainbow trout (*Oncorhynchus mykiss*) the presence of a circadian oscillator in the GIT. Thus, we evaluated daily rhythms mRNA abundance of clock genes (*clock1a*, *bmal1*, *per1*, *cry2* and *rev-erbβ-like*) and gastrointestinal hormones (*ghrelin*, *cck* and *glp1*) along the GIT, and the influence of light and food on such rhythms. Four cohorts of trout were subjected to: 1) Control, 12L:12D with feeding time at ZT3; 2) Light isolation and fasting for 48 h (DD+F); 3) DD and refeeding at CT3 for the last 72h; 4) 12L:12D and feeding at ZT15. Individual samples of foregut, midgut and hindgut were collected in animals from each group every 4 hours along the 24 h cycle for qPCR assays of mRNA abundance of clock genes and GIT hormones. Our results reveal daily rhythmic expression for all the genes and GIT regions. In general, such rhythms persist in the absence of environmental cues, which agree with the existence of a circadian oscillator in trout intestine. Modulators of GIT activity such as ghrelin, CCK and GLP1, appear to subordinate to the circadian system.

Acknowledgements. Funded by the Spanish Agencia Estatal de Investigación and European Fund of Regional Development (PID2019-103969RB-C31) and Xunta de Galicia (Consolidación e estructuración de unidades de investigación competitivas do SUG, ED431B 2019/37) to JLS.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

[ARG8]VASOTOCIN MODULATES PRODUCTION OF PROLACTIN, GROWTH HORMONE AND SOMATOLACTIN IN PITUITARY OF THE TELEOST GILTHEAD SEABREAM (*SPARUS AURATA*): AN *IN VITRO* STUDY

Astola A[1], Galán C[1], Mancera JM[2]*.

[1]Department of Biomedicine, Biotechnology and Public Health, Faculty of Sciences, [2]*Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI·MAR), University of Cádiz, E-11510 Puerto Real, Cádiz, Spain.
*Email: juanmiguel.mancera@uca.es

Arginine vasotocin (VT) is a neurohypophyeal hormone that control synthesis and releases of different adenohypophyseal hormones. This study aims to determine the influence of VT on prolactin (PRL), growth hormone (GH) and somatolactin (SL) production in pituitary of the teleost gilthead seabream (*Sparus aurata*) using an *in vitro* culture approach by assessing expression levels and protein content secreted into the medium. Thirty specimens were sacrificed, and pituitary glands extracted, placed in a 96-well plate with 100 μL of RPMI medium and incubated at room temperature for 24 h. After this time, culture medium was replaced with medium supplemented with VT. Pituitary glands were divided into 3 groups (n = 10) and treated with different doses of VT: i) without VT (control), ii) 10^{-7} M (physiological doses), and iii) 2 times physiological doses. Pituitary glands remained under these conditions for 24 h and then were collected and stored in RNA protective medium. Samples were employed to determine PRL, GH and SL expression using qPCR. In addition, the culture media were also collected after 24 h of treatment and analyzed by dot blot (using specific antibodies against sea bream PRL, GH and SL) to measure the specific hormone secreted. PRL expression was not influenced by any VT treatment, but hormonal content in medium increased when physiological VT doses were employed. Physiological doses of VT decreased both expression levels and GH values in medium compared to control group. However, these parameters enhanced if 2 times physiological VT doses was used. Under this last condition, SL expression as well as hormonal levels in the medium enhanced respect other groups. Our results indicate that it is possible to use *in vitro* approaches to study the synthesis and release of adenohypophyseal hormones by different endocrine factors.

Acknowledgements. Study supported by Proyect PID2020-117557RB-C22.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SILENCING TYROSINE HYDROXYLASES (THS) TO PROMOTE DOPAMINE DEPLETION IN ZEBRAFISH. A POTENTIAL MODEL FOR PARKINSON'S DISEASE

Tormos-Ribes E*, Leal E, Cerdá-Reverter JM.

Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, 12595 Castellon, SPAIN. *Email: elisabethtormos@gmail.com

Dopamine is a key neurotransmitter in vertebrates acting at different levels of the central nervous system (CNS) to regulate a range of functions including emotion, motivation, somatosensory systems, feeding, and reproductive behaviour, among others. Some neurological and psychiatric disorders, such as Parkinson's disease, schizophrenia and drug addiction, are caused by dysfunctional dopaminergic transmission. Dopamine is an intermediate metabolite in the catecholamine synthesis pathway, i.e. norepinephrine/noradrenaline and epinephrine/adrenaline in which tyrosine hydroxylase (TH) is the rate-limiting enzyme catalysing the first step of the biosynthetic pathway (tyrosine to L-DOPA). Subsequently, aromatic L-amino acid decarboxylase (AADC) or dopa descarboxilase (DDC) promotes dopamine synthesis from L-DOPA. Finally, the action of dopamine beta-hydroxylase (DBH) converts dopamine into norepinephrine. Teleost fish have undergone an additional duplication of the whole genome (3R), resulting in additional copies of genes, and the catecolaminergic pathway has been no exception. As a result, TH1 and TH2 paralogs are frequently found in teleost genomes. Both enzymes have similar affinities for the common substrate (Tyr), but have different domains of expression in the CNS, with the expression pattern of TH2 being much more reduced. Mammalian TH knockouts are not viable due to catecholamine deficiency, but not in zebrafish, where DBH knockouts are viable. In this work, we focus on knocking down TH1 and TH2 in zebrafish using CRISPR technologies to generate new strains for the study of somatosensory system and its link to the central motivational pathways. New strains will also be used as a model for Parkinson's disease.

Acknowledgements. This study was financed by funds from MCIN/AEI/ 10.13039/501100011033 (PID2022-136288OB-C33) and, as appropriate, by “ERDF A way of making Europe”, by the “European Union” or by the “European Union Next Generation EU/PRTR”.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

NEUROPEPTIDES REGULATE BIOMINERALIZATION OF BIVALVES SHELLS

Li Z[1]*, Cardoso JCR[1], Peng MX[1], Power DM[1,2,3]*.

[1]*Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. [2]International Research Center for Marine Biosciences, Ministry of Science and Technology, Shanghai Ocean University, Shanghai, China. [3]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China.
*Email: a73168@ualg.pt

Bivalves have hard biomineralized shells and their growth depends on the secretory activity of the mantle, the main shell-building organ. As a first step towards understanding shell production, mantle transcriptomes and proteomes of several bivalves have been generated and a biomineralization toolbox established. Nevertheless, the regulation of mollusc shell formation and more specifically the mantle remains poorly understood. Here, using the symmetric marine bivalve, the Mediterranean mussel (*Mytilus galloprovinciallis*), we identified factors that may regulate shell formation. Using available transcriptomes and proteomes of the mantle we characterized and catalogued neuropeptide precursors and their coding potential and identified putative candidates of mantle-shell regulation. We demonstrate that the Mediterranean mussel mantle has a rich network of fibers and cell bodies, and that its transcriptome is enriched with neuropeptide/peptide hormone precursors. *In vivo* shell damage – repair experiments revealed that the nerve ganglia in the Mediterranean mussel controls mantle neuropeptide gene expression and biomineralized enzyme activity and that shell damage and regeneration are associated with significant changes in the expression of some neuropeptide gene transcripts. We hypothesize that neuropeptides play a critical role in the control of mantle function, including shell growth regulation. Furthermore, among the candidate neuropeptides tested, we found that the calcitonin system is a key regulator of bivalve shell biomineralization and induces shell formation in the mussel, *M. galloprovincialis*. In summary, this study provides histological, molecular and functional data confirming the importance of the nervous system and neuropeptides in the regulation of bivalve shell formation.

Acknowledgements. This study received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 and from the FCT-AGAKHAN/541666287/2019 HealthyBi4Namibe project. ZL was supported by a PhD scholarship from the China Scholarship Council.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

STRESS ATTENUATION BY PLANT EXTRACTS DURING VACCINATION OF JUVENILE SEA BASS (*Dicentrarchus labrax*)

Azizi S, Khansari AR, Tort L*.

Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. *Email: Lluis.Tort@uab.cat

Vaccination is the most powerful and safe prophylactic method to avoid disease outbreaks in fish, but the administration of vaccines can generate an episode of stress and the resulting transient lack of welfare. This work aimed to determine the stress, health, and welfare conditions of fish during this critical point in the production process, and secondly, to determine the attenuation capacity of plant extracts previously administered to the vaccination process.

By measuring plasma cortisol, glucose and lactate levels and also cortisol in skin mucus, the results showed that a product based on carvacrol and thymol was able to reduce the overall stress physiological response, one hour, 24 hours and 21 days after intraperitoneal vaccination. This result was also confirmed by assessing the expression response of genes associated to stress, such as the glucocorticoid receptor, the corticotropin releasing hormone and the heat-shock protein 70. These genes were measured in the skin, intestine and gill tissues and in most cases the extract treatment showed a reduction of the expression levels of vaccinated fish, particularly in intestine and gills. The inflammatory response was also measured through the expression of pro-inflammatory cytokines such as Interleukin-1-b and the results showed an expected increase of this gene in all vaccinated fish groups, but the plant extract prevented the rise of the interleukin in all tissues after 24 hours.

Overall the results indicate that a pre-treatment of carvacrol and thymol plant extract administered just prior a vaccination episode, positively influences the health and welfare of fish under aquaculture conditions.

Acknowledgements. This work was supported by the Project Piscibien funded by the EU-Next Generation Funds under the Plan de Recuperación, Transformación y Resiliencia.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

GLUCOCORTICOSTEROIDS, THE MISSING MOLECULES OF THE BUILDING BLOCKS OF OCTOPUSES' STRESS RESPONSE

Costas C[1]*, Maskrey BH[2]*, Méndez-Martínez L[1], Guerrero-Peña L[1], Tur R[3], García P[3], Touriñan P[3], Canario AVM[4], Scott AP[2], Rotllant J[1]*.

[1]Aquatic Biotechnology Lab, Institute of Marine Research, Spanish National Research Council (IIM-CSIC), 36208, Vigo, Spain. [2]Centre for Environment Fisheries and Aquaculture Science (CEFAS), UK. [3]Pescanova Biomarine Center, O Grove, Pontevedra, España. [4]CCMAR, University Of Algarve, Portugal. *Co-First/Equal authorship. *Email: rotllant@iim.csic.es

Currently, the common octopus (*Octopus vulgaris*) is a promising candidate for diversifying aquaculture in Europe. Its rapid growth, adaptability to captivity, and high nutritional and economic value make it attractive for meeting market demand and reducing pressure on wild populations. However, octopuses' complex characteristics, particularly their sensitivity to environmental factors and need for specific nutritional requirements, present significt challenges for intensive farming. Fortunately, the once-difficult obstacle of high mortality rates during the rearing phase has been overcome, enabling the industrial-scale cultivation of these animals. As interest in octopus aquaculture continues to grow, concerns arise regarding the potential impacts of large-scale production and intensified farming practices on the welfare of the animals. Assessing farm animal welfare typically involves evaluating physical health, immune response, behavior, and physiological indicators, with a particular focus on identifying stress levels. Therefore, understanding how to recognize signs of stress, establish reliable stress biomarkers, and effectively manage stress levels is essential for successful domestication and farming of these creatures. In vertebrates, the production of glucocorticosteroids (GCs) is a key response to stress. These are hormones that regulate energy balance and overactivity of the immune response. Their blood levels are widely used as a reliable indicator of stress in vertebrates. However, the mechanisms underlying the stress response in invertebrates, and especially cephalopods, are still poorly understood. In this study, we demonstrate that octopus hemolymph does not contain either of the major GCs that regulate the stress response in vertebrates. Furthermore, as found in a fish and another mollusk, the blue mussel, we show that the octopus has a poor ability to absorb GCs from the water (<2% over 24h). Therefore, our results indicate that if there is a specific type of hormone produced by octopuses in response to stress, it is neither cortisol nor corticosterone.

Acknowledgement. Project funding: CDTI-IDI-20210907.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

VISUAL AND NON-VISUAL OPSINS REPERTOIRE IN GILTHEAD SEABREAM EMBRYONIC STEM CELLS: A CHRONOBIOLOGICAL APPROACH

Vergès-Castillo A[1]*, Herrera-Pérez P[1], Pendón C[2], Pérez-Sánchez J[3], Martín-Robles AJ[2], Muñoz-Cueto JA[1].

[1]*Department of Biology, Faculty of Marine and Environmental Sciences, INMAR, and [2] Department of Biomedicine, Biotechnology and Public Health, Faculty of Sciences, University of Cádiz, Puerto Real, Spain. [3]Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Spain. * Email: alba.verges@uca.es

Daily and seasonal light-dark cycles represent the most reliable and predictable environmental cue for life on Earth. As a consequence, these periodic changes have exerted selective pressure on organisms, and favour the development of circadian clocks that allow them to track time and anticipate responses to predictable events. This entrainment requires effective light sensing and transduction mechanisms, which in vertebrates are driven by photosensitive cells present in central structures such as the retina and/or the pineal gland. However, in some teleosts as zebrafish, it has been reported that peripheral cells and tissues possess a wide number of opsin photopigments and a light entrainable circadian pacemaker. According to that, in a previous study we have shown that monoclonal embryonic stem cell lines generated from morula stage embryos of gilthead seabream (SAEC-H7) are directly light responsive and exhibit a light-entrainable molecular clock machinery. In the present study, we aimed to identify which opsins are present in SAEC-H7 cells and analyze their daily expression patterns and rhythms under light-dark and constant dark conditions. We shown that seabream embryonic stem cells display a diversity of expressed visual (*sws1*, *sws2a*, *sws2b*, *rh2.4*, *lws*) and non-visual (*opn3*, *opn4m1*, *opn4m2*, *opn4m3*, *opn5*, *opn6a*, *opn7a*, *tmt1a*, *tmt1b*, *tmt2a*, *tmt2b*) opsins, a number of which are under clock-control and as such show robust daily rhythms in expression even under constant dark conditions. With the exception of *lws*, with its acrophase at the beginning of the night, the remaining opsins analyzed peaked at the transition between the end of the night and the beginning of the day. These results reveal that embryonic stem cells have a wide repertoire of visual and non-visual opsins that could sense light and sustain light-entrainable responses from very early stages of development.

Acknowledgements. This research was supported by the Spanish Ministry of Science and Innovation under grant No PID2021-123640OB-C22 (BBLUEAQUA) to JAM-C and AJM-R.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

DAILY RHYTHMS OF LOCOMOTOR ACTIVITY AND EXPRESSION OF NON-VISUAL OPSINS IN THE BLIND MEXICAN CAVEFISH

Conti F[1]*, Vergès-Castillo A[2], Sánchez-Vázquez FJ[1], Muñoz-Cueto JA[2].

[1]*Department of Physiology, Faculty of Biology, University of Murcia, Murcia, Spain. [2]Department of Biology, Faculty of Marine and Environmental Sciences, University of Cádiz, Cádiz, Spain. *Email: javisan@um.es

Most animals and plants possess endogenous circadian clocks that synchronise their physiology and behaviour with the environmental cycles. The most common synchronising signal is the environmental light–dark cycle (LD). Consequently, it can be assumed that animals that evolved in caves, in constant darkness condition, no longer possess a functional light-synchronised biological clock. In this study we used the blind Mexican cavefish, *Astyanax mexicanus*, which represents an excellent model system for the study of numerous adaptive and regressive evolutionary changes (e.g., loss of functional eyes and pigmentation) that have occurred during its evolutionary history. We firstly investigated the photic entrainment and a possible circadian endogenous rhythmicity of this eyeless hypogean species. By using an automatic system based on photocells, we recorded the locomotor activity during different lighting regimes: LD 12:12, 6 hours shift of LD 12:12, constant darkness and continuous dim light. Opsins typically act as light sensors in animals. The opsin family is composed of visual opsins, expressed in retinal photoreceptors, and non-visual opsins expressed in both ocular and extraocular tissues. Considering that previous studies have confirmed that despite the regression of the eyes, juvenile and adult cavefish still show light-dependent responses, the second objective of the study was to investigate the daily expression patterns and rhythms of non-visual extraocular opsins in the *A. mexicanus* brain. Specifically, we quantified the daily transcript levels for a set of non-visual opsin genes by using RT-qPCR and analysed their daily rhythms by cosinor. Results obtained revealed that animals showed an entrainment to the light-dark cycle with a diurnal activity pattern, i.e., increased locomotor activity during daytime and lower locomotor activity during nighttime. We also found significant daily variations and/or rhythmic expression for three genes encoding non-visual opsins (*opn3*, *rgra* and *tmt1b*), which exhibited nocturnal acrophases. Our results suggest that daily rhythms in extraretinal non-visual opsins are still able to transduce daily photic cycles and could be sustaining behavioural and other light-entrained rhythms in blind cavefish species.

Acknowledgements. This research was supported by the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant No 956129 (EasyTRAIN) to FJS-V and by the Spanish Ministry of Science and Innovation under grants No PID2021-123640OB-C21 and PID2021-123640OB-C22 (BBLUEAQUA) to FJS-V and JAM-C, respectively.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

PHYSIOLOGICAL COLOUR CHANGES IN ECOTOXICOLOGY

Knigge T[1], Gouveneaux A[2], Hue S[1,2,3], Chabenat A[1,2,3], Bellanger C[2,3], Ford A[4].

[1]Normandie Univ, UNILEHAVRE, FR CNRS 3730 Sciences Appliquées à L'Environnement, UMR-I02, Environmental Stress and Biomonitoring of Aquatic Environments, University of Le Havre Normandy, Le Havre, France. [2]Normandie Univ, UNICAEN, CNRS EthoS UMR 6552, 14000 Caen, France. [3]Univ Rennes, CNRS, EthoS (Éthologie animale et humaine) - UMR 6552, F-35000 Rennes, France. [4]Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Portsmouth, Hampshire PO4 9LY, UK.

*Email: thomas.knigge@univ-lehavre.fr

Rapid physiological colour changes allow animals to conceal themselves and to adapt to their surrounding environments. Colour changes in cephalopods, such as the European cuttlefish, *Sepia officinalis*, provide a particularly impressive example of camouflage. But physiological colour changes can also be found in many other species, such as juvenile green crabs, *Carcinus maenas*, or sand shrimp, *Crangon crangon*. These colour changes can be controlled either nervously, as in cephalopods, or neurohormonally, as in crustaceans. Both, neuronal and neurohemal control are influenced by neurotransmitters, such as serotonin or dopamine. Environmental pollutants have the potential to interfere with this signalling cascade, thus disturbing physiological colour changes and cryptic behaviour. Consequently, chromatophore movements and colour changes may serve as indicators of environmental pollution and could point to detrimental effects on behavioural ecology that are important for the animals' survival. Colour change bioassays, therefore, provide a promising tool for environmental testing with the advantage of being non-invasive, inexpensive and relatively straightforward.

Acknowledgements. This project was supported by French-English Interreg projects 4a PeReNE (#4230) and 5a RedPol (# 185).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TRANSCRIPTION LEVELS OF GNRH AND SEROTONIN SIGNALING GENES IN THE TISSUES AND DURING GAMETOGENESIS OF THE MEDITERRANEAN MUSSEL (*MYTILUS GALLOPROVINCIALIS*)

Leonida JI[1,2]*, Diaz de Cerio O[2]*, Ortiz-Zarragoitia M[2]*.

[1]Institute of Marine Fisheries and Oceanology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines. [2]CBET Research Group, Dept. Zoology & Animal Cell Biology, Faculty of Science & Technology and Research Centre for Experimental Marine Biology and Biotechnology (PiE–UPV/EHU), University of the Basque Country (UPV/EHU), Areatza Hiribidea 47, 48620, Plentzia, Basque Country, Spain. *Email: maren.ortiz@ehu.eus

Mussels are among the most ubiquitous bivalves with great economic and ecological significance. However, the fundamental aspects of their reproductive endocrinology are not fully elucidated, which can be primarily attributed to their primitive organ system and undefined signaling pathways. Hence, their decentralized nervous system is expected to fulfill the primary regulatory functions that control their reproduction and other physiological activities. GnRH-like neuropeptides identified in invertebrates were reported to have diverse functions other than reproduction, whereas the serotonin (5-HT) neurotransmitter system is one of the well-conserved signaling systems that were transcriptomically investigated for their presumed reproductive roles in bivalves but not in the Mediterranean mussel *Mytilus galloprovincialis*. This study aimed to analyze the genes involved in these signaling systems and evaluate their involvement in the reproduction of Mediterranean mussel populations in the Basque coast. Identification and cloning of partial sequences of the GnRH receptor (*gnrhr*), tryptophan hydroxylase (*tph*, serotonin synthesis-limiting enzyme), and serotonin receptor (*5-htr*) genes were performed using the mussel ganglia and gonads. Transcriptomic analysis (qPCR) revealed generally higher expression levels of the sequenced reproductive gene markers in the three ganglia (i.e. cerebral, visceral, and pedal ganglia) compared to the peripheral tissues, confirming that the nervous system fulfills the key neuroendocrinological regulations in mussels. Expression levels of the reproductive genes showed a consistent upregulation during the onset of oogenesis and spermatogenesis followed by a constant downregulation until reaching sexual maturity. This trend implies the involvement of these genes in the initial phases of gametogenic development. The gradual elevation in the transcription of *tph* and *5-htr* during the spawning stage coincides with the widely reported spawn-inducing activity of serotonin in bivalves. A consistent sex-specific variability leaning toward the higher transcription of both serotonergic genes in male tissues was also observed.

Acknowledgements. Work funded by the Basque Government (Grants to consolidated research groups IT1743-22).

SESSION 3.
ENVIRONMENTAL ALTERATIONS AND BIOLOGICAL ANSWERS/IMPACTS

PLENARY LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TIME TO ACT: PROTECT MARINE LIFE FROM ENDOCRINE DISRUPTING POLLUTANTS

Pinto PI[1]*, Miglioli A[2], Baumann L[3], Baynes A[4], Blanc-Legendre M[5], Cancio I[6], Cousin X[5], Dumollard R[2], Ford A[7], Knigge T[8], Minier C[8], Monteiro M[9], Sturve J[10], Monsinjon T[8], Power DM[1], Katsiadaki I[11]

[1]*Centro de Ciências do Mar (CCMAR), Universidade do Algarve, 8005-139 Faro, Portugal. [2]Observatoire Océanologique de Villefranche sur Mer (VFM), 06230 VFM, France. [3]Amsterdam Institute for Life and Environment, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, Netherlands. [4]Centre for Pollution Research and Policy, Brunel University London, Kingston Lane, Uxbridge, Middlesex, UB8 3PH, UK. [5]MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, INRAE, Palavas, France, 34250 Palavas-Les-Flots, France. [6] Plentzia Marine Station/University of the Basque Country (PiE-UPV/EHU), Areatza 47, 48620 Plentzia, Basque Country, Spain. [7]Institute of Marine Sciences, University of Portsmouth, Portsmouth, Hampshire PO4 9LY, UK. [8]Environmental Stress and Aquatic Biomonitoring (SEBIO), Université Le Havre-Normandie, 76600 Le Havre, France. [9]CESAM & Dbio, Universidade de Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal. [10]University of Gothenburg, BIOENV, 40530 Gothenburg, Sweden. [11]Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Dorset DT4 8UB, UK. *Email: ppinto@ualg.pt

Our oceans harbour a vast and understudied biodiversity that is threatened by climate change, pollution, and other global issues. More than 80% of domestic and industrial effluents enter the oceans raw leading to the accumulation of harmful pollutants, including endocrine-disrupting chemicals (EDCs), in marine ecosystems. Following two Euromarine funded MODEL-EDC workshops where >70 experts met, the current knowledge gaps about the endocrine system and endocrine disruption across marine animal-taxa and the tools and monitoring framework needed to better protect marine life were reviewed. The results presented summarise the outcome of bibliographic reviews, which identified the increasing knowledge on EDCs effects produced in recent decades. Strong biases were identified in the scope of the research that was mostly focussed on terrestrial and/or freshwater organisms (mostly vertebrates), whilst some misinterpretations were identified due to application of concepts in vertebrate endocrinology to non-vertebrate taxa. In addition, the significant knowledge available for a minority of marine taxa (mainly vertebrates) was restricted to relatively few endocrine pathways. In general, the endocrinology of invertebrates is poorly studied, and EDC hazard assessment is inadequately covered by international regulatory tests or monitoring despite their key role in ecosystem functioning. Recommendations include an increased and improved organization of knowledge across marine phyla, the combination of genomic resources, cross-species extrapolation tools and expert knowledge to prioritise neglected species or endpoints to use in chemical testing, better interactions between key research disciplines and translation to regulators and environmental monitoring programs. Inspired by the UN Oceans Decade, this presentation highlights the need for action to protect marine life from the impacts of chemicals, especially EDCs, considering the breath of marine biodiversity.

Acknowledgements. These results were supported by Euromarine through Foresight Workshop funding (2019 call) and FCT funds to CCMAR (UID/Multi/04326/2021).

ORAL COMMUNICATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CYPRINODON VARIEGATUS TRANSCRIPTOMIC ALTERATIONS CAUSED BY ENDOCRINE DISRUPTION COMPOUNDS AND COMPOSTABLE PLASTIC BAG LIXIVIATES

Schonemann AMS[1,2]*, Beiras RB[1,2]

[1]*Centro de Investigación Mariña da Universidade de Vigo (CIM-UVigo), Vigo, Galicia, Spain. [2]Department of Ecology and Animal Biology, University of Vigo, Vigo, Galicia, Spain. *Email: alemartinez@uvigo.gal

Plastic is a heterogeneous group of substances that are present globally in different ecosystems and in different forms, decomposition stage and size, conditioning the associated risk for the ecosystem. In aquatic environments, the migration to the water of plastic additives that are embedded in the base polymer supposed an extra vector of exposure for aquatic organisms. Several plastic additives are known to cause endocrine disruption and the number of suspected endocrine disruption substances is increasing as industrial innovations produce new substances. New biodegradable or compostable plastics are appearing, composed of a complex mix of additives. Addressing this situation, a transcriptomic approach has been taken to evaluate firstly, the molecular response of the marine fish model *Cyprinodon variegatus* to an estrogenic endocrine disruption (ethinyl estradiol, EE2 100 ng/L) and to an antiandrogenic endocrine disruption (Di(2-ethylhexyl)phthalate, DEHP 5 µg/L). Secondly, the toxicity of lixiviates of 1 g/L of three different plastic bags has been tested: two compostable bags (P045 “Green-Materbi®” and P072 “BagBrown”) and a low-density polyethylene bag (P017 LDPE). RNAseq results show that the EE2 reports a widespread transcriptome alteration, showing a statistically significant change on the abundance of 1224 transcripts, among them known biomarkers of estrogenic endocrine disruption as vitellogenin, zona pellucida, or wap-like. DEHP altered the abundance of 773 transcripts, upregulating genes related with protein transport among others and downregulating genes related with serine-type endopeptidase activity, a process upregulated by the estrogenic treatment. In the other hand, plastic bag lixiviates report alterations not related with estrogenicity, founding in the compostable bags “Materbi” and “BagBrown”, 1160 and 350 altered transcripts respectively and in the LDPE, 648 altered transcripts.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CAN LIQUID TISSUES RECAPITULATE EPIGENETIC INFORMATION IN DIFFICULT-TO-ACCESS INTERNAL TISSUES?

Mustapha UF*, Piferrer F.

Group of Biology of Reproduction (GBR), Department of Renewable Marine Resources, Institut de Ciències del Mar (ICM), Consejo Superior de Investigaciones Cientificas (CSIC), Passeig Marítim, Barcelona, Spain.
*Email: umarfk@icm.csic.es

Studying epigenetic modifications of internal tissues such as the brain, liver, and gonads usually requires sacrificing the animal under investigation. Therefore, identifying surrogate tissues could enable a non-invasive and non-lethal means to elucidate epigenetic modifications reflecting target tissues. This would also enable repeated measures and thus real-time tracking of environmental perturbation physiological status or disease progression. Here, we reviewed the current knowledge on the potential use of liquid tissues such as blood, sperm, and hemolymph as surrogates for epigenetic modifications in difficult-to-access internal tissues. We conducted a systematic review of the literature using the Web of Science Core Collection database as a source. The search was carried out following the PRISMA flowchart method. A total of 82 papers including reviews (10) were analyzed. There were more papers on epigenetic modifications in internal tissues and blood (66), followed by sperm (5) and hemolymph (1). Fifty-two (79%) of the studies on internal tissues and blood were done in humans 10 (15%) in mice and 4 (6%) in birds. Three (60%) and 2 (40%) of the studies on sperm were in mice and cattle, respectively, whereas the study on hemolymph was done in bees. Thirty-two papers on humans and four on mice reported epigenetic modifications between blood and internal tissues, while one paper on cattle compared the modifications between sperm, brain, and mammary glands while no comparison was made in the hemolymph paper. Analysis of five studies examining seven different human tissues and blood in different conditions such as aging and diseases revealed ~2,000 differentially methylated CpGs (DMCs) per condition, of which about 10% were shared between blood and the internal tissue. The amount of overlap varied among tissues, from ~1% in adipose tissue to ~13% in the brain and kidney. Interestingly, most (~80%) of these shared DMCs were consistently hyper-or hypomethylated in the blood and the other tissue under examination. Similarly, a study focusing on Cattle sperm and somatic tissue DNA methylation revealed a total of 22 (68%) overlapped DM genes (each gene has ≥ 5 CpGs), of which 20 (91%) were either hyper-or hypomethylated. Taken together, we conclude that blood methylation changes have the potential to mirror methylation changes occurring in various internal tissues. Sperm methylation changes also look promising but more data is needed for verification. However, with the data available at present, the potential use of hemolymph as a surrogate is still unclear. Therefore, we demonstrate that liquid tissues have the potential to inform epigenetic changes occurring in internal tissues.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TOWARDS A BETTER CONSIDERATION OF ENDOCRINE DISRUPTION WITHIN THE TECHNICAL GUIDANCE FOR DERIVING ENVIRONMENTAL QUALITY STANDARDS

James A[1], Kroll A[2], Minier C[3].

[1]INERIS, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France. [2]Oekotoxzentrum, c/o Eawag–Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland. [3]UMR-I 02 INERIS-URCA-ULHN SEBIO, Normandie University, FR CNRS 3730 Scale, 76063 Le Havre Cedex, France.

Endocrine-disrupting chemicals (EDCs) are a reason for growing concern because of their substantial and long-lasting deleterious effects on human health and wildlife populations. These include direct effects on aquatic organisms and may be a concern to species feeding on the aquatic food chains and water, including humans. In the European Community, the dedicated legislative tools to protect the aquatic environment and human health from contaminants released to surface waters is the Water Framework Directive (WFD). The achievement of protection goals is assessed through the comparison of concentrations measured in the media and thresholds of no effect called Environmental Quality Standards (EQSs). As EDCs are explicitly mentioned in the WFD, an analysis of the state of the art was undertaken on how far and how consistently ED properties were considered in the derivation of EQS values. Our results reveal substantial heterogeneity according to substance and that among substances with ED evidences, EQSs have been derived without considering ED properties for 70 % of them. A methodology to better consider endocrine disrupting properties is proposed and includes a logical and systematic approach to derive EQSs with a proposal to specify additional assessment factors based on the specific hazard and potential uncertainty.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

THE DOLPHIN AND THE WHALE: FROM THE PAST TO THE ANTHROPOCENE

Castro L Filipe C[1,2]*, Oliveira D[1,2], Valente R[1,2], Fonseca E[1], Santos M[1,2], Ruivo R[1].

[1]Deparment of Biology, Faculty of Sciences, University of Porto, Porto, Portugal. [2]CIMAR/CIIMAR, University of Porto, Matosinhos, Portugal. *Email: filipe.castro@ciimar.up.pt

Animal genomes encompass a network of gene families and pathways central for endocrine homeostasis. Importantly, an orchestrated set of these molecular components takes part in the response against toxicants – the so-called Chemical Defensome (CD). Yet, why some animal species are more sensitive than others to man-made chemicals remains elusive. In effect, animals exhibit conserved and divergent sensitivities to external stimuli. The contribution of evolutionary processes in the configuration of critical endocrine components underlying such dissimilar responses is far from fully assessed. Here, we investigate the evolution of two central molecular nodes of the CD: the Nuclear Receptors and the Flavin-containing Monooxygenase (FMOs) in Cetacea species. We combine extensive comparative genomics, phylogenetics, and functional assays to decipher the evolution of endocrine systems and their impact by man-made chemicals. We show the vital influence of mutation, gene duplication and gene loss at key gene families of the Chemical Defensome as powerful drivers of evolutionary change, with impacts at the endocrine disruption level. Our approach puts into context the role of evolution in endocrine disruption processes, which would be impossible to reveal with model species.

Acknowledgements. This work is a result of the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by NORTE 2020, under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

NON-INVASIVE TECHNOLOGY TO DETECT STRESS BIOMARKERS IN FISH

Cartan S[1]*, Jerez-Cepa I[1], Rodriges-Teixeira S[2], Martos- Sitcha JA[1], Mancera JM[1].

[1] *Department of Biology, Faculty of Marine and Environmental Sciences, Institute of Marine Research (INMAR), University of Cádiz, Puerto Real, Spain.[2] Tyndall National Institute, University College Cork, Cork, Ireland.
* Email: sara.cartan@uca.es.

The growth of aquaculture has led to increasing interest in farmed fish welfare. Detecting stress situations that activate the hypothalamic–pituitary-interrenal axis are mandatory to improve animal well-being. Plasma cortisol levels have been used as a stress indicator; however, it is necessary to develop less invasive techniques in non-invasive alternative matrices such as mucus or water. Currently, the most common method to measure cortisol from fish tissues is ELISA, but its use can be problematic due to cross-reactivity and low sensitivity. Furthermore, its determination in some matrices requires a tedious sample preparation. Electrochemical immunosensors are presented as an alternative to measure cortisol directly and in different matrices by measuring electrochemical properties changes of a conductive material as a response to an antigen-antibody binding. By this technique, cortisol can be measured to concentrations down to 40 pg mL^{-1}. The electrochemical immunosensors of this study were develop on Tyndall National Institute (Cork, Ireland). To validate them, seabass juveniles, were exposed to two stress conditions: i) air exposure (3 min); and ii) crowding (1 h) with periodical chasing every 15 min. For the first condition, plasma and mucus samples were taken 10, 30, 60 min and 24 h after air exposure. Water samples were periodically taken during the first hour post-stress. In the second condition, plasma and mucus samples were immediately taken after the stress situation. Water samples were taken at different time points during the stress induction. Samples were also taken in a non-stressed group. The preliminary results are in agreement with cortisol levels determined by other techniques. Promising correlations between water levels and the other matrices (plasma and mucus), validate the potential of electrochemical immunosensors to assess fish welfare in a less invasive way.

Acknowledgements. Improving Green Innovation for the Blue Revolution (IGNITION). Co-funded by the European Union (Grant Agreement 101084651).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TRACING LIFE HISTORY OF THICKLIP GREY MULLETS INHABITING ESTUARIES WITH DIFFERENT XENOESTROGENIC PRESSURES ALONG THE SOUTHERN BAY OF BISCAY

Nzioka A[1]*, Valencia A[1], Rojo-Bartolomé I[1], Lekube X[1], Ortiz-Zarragoitia M[1], Madeira MJ[2], Méndez-Vincente A[3], Correia AT[4,5,6], Diaz de Cerio O[1], Cancio I[1].

[1]*CBET Research Group, PiE-UPV/EHU, University of the Basque Country (UPV/EHU), Areatza Hiribidea s/n, 48620 Plentzia, Basque Country, Spain. [2] SystBioGen Research Group, Faculty of Science & Technology and Lucio Lascaray Research Centre, UPV/EHU. Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain.[3] Servicios Científico-Técnicos, Unidad de Ensayos Medioambientales. Universidad de Oviedo, Campus de Mieres, Gonzalo Gutierrez Quiros S/N, 33600 Mieres, Spain. [4] CIIMAR, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N 4550-208 Matosinhos, Portugal. [5] ICBAS, Universidade do Porto (UP), Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. [6] DeBA, ECVA, Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta de Prados, 5001-801 Vila Real, Portugal. *Email: anthony.nzioka@ehu.eus

In this study, the life history of the thicklip grey mullet (*Chelon labrosus*) was examined to elucidate whether the high intersex ratios observed in different estuaries were linked to their life history. Mullets, although capable of withstanding pollution, are sensitive to xenoestrogens that disrupt gonad development, sometimes leading to intersex condition. This effect has been detected in high percentages in the Urdaibai estuary in Gernika due to the malfunctioning of its WWTP. To understand the xenoestrogen exposure history of these fish which inhabit estuaries but reproduce at sea, we need to know whether marine reproductive migration results in return to the polluted estuary of origin. For mullets from polluted estuaries, are they linked geographically for life to xenoestrogens? The prevalence of intersex in Gernika since 2007, when it was at its highest (70%), decreased during 2014-17. However, prevalences are picking back, reaching a new highest in 2021 (47%). The prevalence in the closest estuary of Plentzia is 0. The mullet migration patterns reconstructed from otolith core-to-edge elemental profiles revealed two distinct life histories: estuarine-residents and marine-migrants. In Gernika, otolith analysis of 30 individuals showed that 83% of them could be considered resident, 20% making repeated movements from estuaries to the sea. In Plentzia, only 53% were considered residents. Furthermore, otolith shape and elemental analyses showed significant differences between Gernika and Plentzia suggesting a lack of connectivity. In contrast, microsatellite analysis of mullets inhabiting five Basque estuaries plus two outgroups from Cadiz and Greece concluded that they form a panmictic population. Thus, results suggest that adult mullets forming a single genetic population from the Bay of Biscay to the Mediterranean show fidelity to the estuary of the first recruitment and therefore xenoestrogen exposure in Gernika resulting in intersex testes would be life-long.

Acknowledgements. This work was funded by the Spanish MICIN and EU-FEDER/ERDF (PGC2018-101442-B-I00), the Basque Government (IT1302-19 and IT1743-22), a TA project of the Horizon-2020 project ASSEMBLE PLUS. 730984 and by the Strategic Funding UID/Multi/04423/2019 through national funds provided by FCT and ERDF program PT2020. A.N. is a recipient of a pre-doctoral grant from UPV/EHU.

POSTER PRESENTATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

POTENTIAL INDICATORS OF CHRONIC STRESS IN FISH

Leal E.[1*], Tormos E.[1], Beltrán X, Cerdá-Reverter JM.[1]

[1]Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, Torre la Sal s/n, Ribera de Cabanes, 12595 Castellon, Spain

*Email: esther@iats.csic.es

Currently, one of the main problems in fish farming, especially with regard to new species, is the response of the animals to stressful situations associated with the farming conditions. Stress can be defined as a state of threat to the homeostasis of an organism, which tries to restore itself by activating a complex repertoire of behavioral and physiological processes that are integrated in its adaptive response. This response includes the synthesis and secretion of adrenal glucocorticoids. In fish, as in humans, the major glucocorticoid is cortisol and its elevation is one of the most conserved responses to stress. For this reason, the determination of its levels in the plasma is an indicator of the degree of stress experienced by the organism. However, plasma levels do not necessarily reflect states of chronic stress, as prolonged exposure to stressors induces desensitization of the HPI axis to the stressor as a result of allostatic overload. Recent studies have shown that cortisol accumulates in external structures such as hair, feathers or scales in vertebrates. Measuring cortisol in scales could therefore be a good indicator of cumulative or chronic stress or, in other words, an excellent way of obtaining a history of non-specific stressful events. Our preliminary studies show significant cortisol accumulation in farmed species such as sea bass, sea bream, grouper and amberjack. However, the highest cortisol accumulation was found in the fins and no zonation of cortisol accumulation levels was observed in the scales.

Acknowledgements. This study was financed by funds from MCIN/AEI/ 10.13039/501100011033 (PID2022-136288OB-C33) and, as appropriate, by “ERDF A way of making Europe”, by the “European Union” or by the “European Union Next Generation EU/PRTR”

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

REGULATION OF THE FISH STRESS RESPONSE TO A CHRONIC EXPOSURE OF EMERGING CONTAMINANTS

Ruiz N*[1], Blonç M[1], Llorca M, Farré M, Tvarijonaviciute A[4], Tort L[1], Teles M[1,2].

[1]Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. [2] Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. [3]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEACSIC, Barcelona, Spain. [4] Interdisciplinary Laboratory of Clinical Analysis Interlab-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia 30100, Spain. *Email: Nuria.Ruiz.Iglesias@uab.cat

Emerging contaminants are environmental wastes of concern, as not only are accumulated in increasing amounts, but they may cause negative effects on the aquatic biota. The present work has focused on whether the stress response is modulated by two of these emergent contaminants, gemfibrozil (a commonly prescribed pharmaceutical lipid regulator) and nanoplastics. Their constant use and discard combined to their environmental persistence and poor removal rates from wastewater makes of these emergent contaminants ubiquitous in aquatic systems. We assessed the effects in hematological, biochemical, oxidative stress and gene expression response of a 28-day waterborne exposure to both an environmentally relevant concentration and a spiked concentration in juvenile fish. Bioaccumulation of these compounds in liver and muscle, as well as possible variations on haematological parameters, blood plasma biochemistry, and gene expression were investigated. The results indicated that both gemfibrozil and nanoplastics accumulated in both liver and muscle. Moreover, significant differences were observed in hematological variables. Regarding plasma cortisol, the response was different depending on the contaminant, since gemfibrozil showed a reduction of plasma cortisol probably associated to its metabolic interaction with the lipid synthesis pathways and therefore, cortisol production. This mechanism was supported by changes observed in the genetic markers of lipid metabolism. Although the cortisol response is different between the two contaminants, gene expression analysis corroborates the biochemical effects of both nanoplastics and gemfibrozil. Overall, the results from the present study first suggest that bioaccumulation of emergent contaminants trigger several reactive responses including the activation of the hypothalamic-pituitary-interrenal axis, and second, that some of them such as gemfibrozil, may interfere with the regulation of the cortisol response.

Acknowledgements. This research was funded by MICIN/ AEI/10.13039/501100011033/ “FEDER Una manera de hacer Europa”, grant number PID-2020-117557RB-C21”.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

METABOLIC AND ENDOCRINE EFFECTS OF A CHRONIC EXPOSURE TO THE LIPID REGULATOR GEMFIBROZIL IN GOLDFISH (*CARASSIUS AURATUS*)

Ruiz N*[1], Blonç M[1], Llorca M, Farré M, Tvarijonaviciute A[4], Tort L[1], Teles M[1,2].

[1]*Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. [2]Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. [3]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEACSIC, Barcelona, Spain. [4] Interdisciplinary Laboratory of Clinical Analysis Interlab-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia 30100, Spain. *Email: Nuria.Ruiz.Iglesias@uab.cat

Lipid regulators, such as fibrates, are pharmaceuticals manufactured to treat dyslipidemias in humans. Their constant use and discard combined to their environmental persistence and poor removal rates from wastewater makes of these emergent contaminants ubiquitous in aquatic systems, with gemfibrozil being the most detected fibrate in water. For this reason, the present study aimed to assess the effects in hematological, biochemical, oxidative stress and gene expression response of a 28-day waterborne exposure to both an environmentally relevant concentration (1.5µg/L) and a spiked concentration (15mg/L) of gemfibrozil in adult individuals of the model organism Carassius auratus. To this end, bioaccumulation of this compound in liver and muscle, as well as possible variations on haematological parameters, blood plasma biochemistry, and liver gene expression were investigated. The results indicated that, following exposure to the highest concentration of gemfibrozil, this compound accumulated in both liver and muscle. Similarly, significant differences were observed in haemoglobin levels, and mean corpuscular haemoglobin concentrations in individuals exposed to 15mg/L of gemfibrozil. The biochemical profiling of blood plasma revealed significant decreases of glucose and cortisol levels with exposure to gemfibrozil, as well as a significant increase in the ferric reducing ability of plasma (FRAP). Lastly, real-time qPCR analyses indicated significant upregulation of genes related to antioxidant defences (i.e. gpx, gst) and lipid metabolism (i.e. apoa1). Other genetic markers of lipid metabolism (i.e. pparβ and pparY) displayed an increasing trend with increasing gemfibrozil concentrations, although below the threshold for statistical significance. Overall, the results from the present study suggest that bioaccumulation of gemfibrozil in C. auratus alters, to some extent, the metabolism of lipids and triggers antioxidant mechanisms.

Acknowledgements. This research was funded by MICIN/ AEI/10.13039/501100011033/ “FEDER Una manera de hacer Europa”, grant number PID-2020-117557RB-C21”.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

DYNAMIC EVOLUTION OF TEMPERATURE RECEPTOR TRPV (TRANSIENT RECEPTOR POTENTIAL VANILLOID) FAMILY

Morini M[1,2], Bergqvist CA[3], Asturiano JF[1]*, Larhammar D[3], Dufour S[2].

[1]*Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València. Camino de Vera s/n, 46022. Valencia, Spain. [2]Laboratory BOREA, MNHN, CNRS, Sorbonne University, IRD, Paris, France. [3]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden.
*Email: jfastu@dca.upv.es

The IPCC reports that ocean surface temperature will increase by 1 to 3 °C over the next 80 years. Fish are particularly vulnerable to temperature changes. Among various sensory and physiological functions, the TRPV family of ion channels plays a crucial role in thermosensing and temperature-dependent regulation. This research aimed to investigate the number, origin and evolution of TRPV among vertebrates, with a special focus on the impact of whole genome duplications (WGD), gene-specific duplications and gene losses in “fish”. Gene searches, phylogenetic and syntenic analyses revealed a larger number of TRPV genes in vertebrates than currently assumed, with three additional TRPV types in the ancestor of gnathostomes, TRPV7, 8 and 9. Evolutionary scenario shows that five TRPV types (TRPV1, 4, 5, 7, 8) existed in the vertebrate ancestor after the two rounds of WGD and one local gene duplication before the divergence of cyclostomes and gnathostomes. TRPV7 and 8 were lost independently in various vertebrate lineages such as in actinopterygians before the emergence of holosteans, in sauropsids, and in mammals before the emergence of eutherians. TRPV3 and 9 originated from local duplications of TRPV1 and are present in extant elasmobranchs, while TRPV9 was lost in the osteichthyan ancestor and TRPV3 in the actinopterygian ancestor. TRPV2 arose by local duplication of TRPV1 in the tetrapod ancestor. Duplications of TRPV5 occurred independently in various lineages such as chondrichthyans, anurans, sauropsids, mammals, Polypteridae and Esocidae. After the teleost-specific WGD, TRPV1a was retained in all teleosts and TRPV1b in some, whereas a single paralog was retained for TRPV4 and 5. The salmonid-specific WGD duplicated TRPV1a, 4, and 5. This study provides a comprehensive evolutionary scenario for the vertebrate TRPV family, proposing a classification of TRPV across vertebrates.

Acknowledgements. Supported by MCIU (EELGONIA; RTI2018-096413-B-I00), European Union NextGenerationEU (PRTR-C17.I1) and Generalitat Valenciana (THINKINAZUL/2021/012) to SEASPERM.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

BREAKDOWN NANOPLASTICS ARE ACCUMULATED IN ZEBRAFISH AFTER A LONG-TERM EXPOSURE BUT DO NOT ALTER CORTISOL LEVELS NOR CHANGE mRNA TRANSCRIPTION OF VITELLOGENIN

Brandts I[1,2], Franco-Martínez L[3], Tvarijonaviciute A[3], Llorca M[4], Farré M[4], Teles M[1,5]*.

[1]Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. [2]Institute of Marine Sciences (ICM-CSIC), Pg. Marítim de la Barceloneta 37–49, 08003, Barcelona, Spain. [3]Interdisciplinary Laboratory of Clinical Analysis INTERLAB-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia 30100, Spain. [4]Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain. [5]Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. *Email: mariana.teles@uab.cat

Large amounts of plastic waste enter aquatic environments every year. Plastic objects degrade into nanoplastics (NPs, < 1 µm), and these particles have become a potential threat to aquatic ecosystems. Knowledge on the effects of manufactured NPs is increasing, but information regarding the toxicity of breakdown NPs remains elusive. Moreover, there is no available information concerning the potential endocrine disruption potential of these particles in fish. Changes in the hypothalamus–pituitary–interrenal (HPI) axis function, measured as cortisol levels, are important indicators of fish condition.. High cortisol levels mobilize and elevate glucose production in fish through glycogenesis and glycogenolysis to handle the energy request generated by the stress agent for the “fight or flight” reaction. In this study, zebrafish (*Danio rerio*) were exposed to model polystyrene (PS)-NPs and to mechanically broken-down high-density polyethylene (HDPE)-NPs for 28 days. Following, fish were sacrificed with an anesthetic overdose and whole-body levels of NPs, cortisol and glucose levels were measured. Energetic and oxidative stress markers were measured in liver, as well as mRNA levels of genes related involved in lipid-metabolism and reproductive functions. Results showed that only polyethylene levels, corresponding to the breakdown-HDPE particles, appeared to be accumulated in exposed fish, but PS-NPs were not accumulated. With respect to the stress markers, no changes were found cortisol nor glucose whole-body levels, however, glucose levels were decreased in the liver of fish exposed to PS-NPs. No changes were found in molecular markers for either of the NPs. Altogether, results suggest that results suggests that there could be a disturbance in the energy balance of *D. rerio* after 28 days of exposure to PS-NPs, but no apparent interference with the HPI axis.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

NANOPLASTICS ARE ACCUMULATED IN THE GUT AND BLOOD OF FISH AND INDUCE A DECREASE IN PLASMA CORTISOL LEVELS

Brandts I[1,2]*, García-Meilán I[3], Lima J[4], Llorca M[5], Farré M[5], Tvarijonaviciute A[6], Tort L[1], Teles M[1,7].

[1]Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain.[2]Institute of Marine Sciences (ICM-CSIC), Pg. Marítim de la Barceloneta 37–49, 08003, Barcelona, Spain. [3]Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain. [4]Institute of Bioscience, Department of Physiology, Universidade de São Paulo, Brazil. [5]Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain. [6]Interdisciplinary Laboratory of Clinical Analysis INTERLAB-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia 30100, Spain. [7]Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. *Email: irene.brandts@uab.cat

Nanoplastics (NPs) are plastic particles of less than 1 µm in size formed in the environment by the degradation larger plastic waste. These particles can cross biological barriers, such as the gastrointestinal and brain blood barriers, which could make them the most dangerous among plastic debris. Studies on the effects of NPs have increased in recent years, with findings ranging from metabolism disruption to developmental and behavioural problems. However, very little information exists on their endocrine disrupting potential. The main objective of the present work was to evaluate if polystyrene-NPs (PS-NPs) are recognized as a stressor by the hypothalamus-pituitary-interrenal (HPI) axis of rainbow trout (*Oncorhynchus mykiss*) by measuring plasma cortisol levels. For this purpose, adult rainbow trout (50.2 g mean weight) were orally intubated with PS-NPs (44 nm, 100 µg/L, 1 mL per fish). After 96 h fish were sacrificed, and blood, liver and gut were sampled. Cortisol, intermediary and lipid metabolism (glucose, cholesterol, and triglycerides) biomarkers were determined in plasma after PS-NPs exposure. Additionally, quantification of PS-NPs levels was carried out in blood, gut and liver of fish. Histology was done in the gut to assess damage. The results of the present study revealed that PS-NPs were detected and quantified in both blood and gut of exposed fish, but not in liver. Plasma cortisol levels showed a significant decrease in PS-NPs exposed fish when compared to control group. In fish, cortisol is involved in the stress response as well as in many aspects of the endocrine-mediated immune response and therefore the observed decrease could suggest an impairment of the HPI axis. Triglycerides levels in plasma were increased in the exposed individuals, which could point to altered lipid metabolism, potentially affecting the energetic status of rainbow trout. No histological alterations were found in gut.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

EFFECT OF SEAWATER pH AND TEMPERATURE ON SPERM MOTILITY OF AQUACULTURE MARINE FISH SPECIES

Pérez LM[1]*, González-López WA[1], França TS[1], Sanchez MP[1], Fernández-García F[1], Morini M[1], Mañanós E[2], Felip A[2], Gómez A[2], Duncan N[3], CalduchJ[2], Holhorea PG[2], Belenguer A[2], Pérez-Sánchez J[2], Asturiano JF[1].

[1]Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal, Universitat Politécnica de Valencia. Edicicio 7G. 46022 Valencia, Spain. [2]Instituto de Acuicultura de Torre de la Sal, CSIC. Ribera de Cabanes 12595, Castellón, Spain. [3]Institut de Recerca y Tecnología Agrolimentaria (IRTA) Sant Carles de la Ràpita. Carretera de Poble Nou, km 5.5 43540 Sant Carles de la Ràpita, Tarragona, Spain.

*Email: mlpereig@dca.upv.es

The climate change entails a decrease in seawater pH, and an increase in its temperature. It is possible that the marine fish sperm cells, which are released to the sea at spawning, can be negatively affected by these changes on the water. For that reason, different seawater pH and temperatures have been tested in the sperm of 4 aquaculture marine species: the European eel (*Anguilla anguilla*), the European sea bass (*Dicentrarchus labrax*), the gilthead seabream (*Sparus aurata*) and the Senegalese sole (*Solea senegalensis*). Their sperm motility parameters have been analyzed by a CASA-mot system. In the eel, seawater pH values lower than 7.8 or higher than 8.2 caused lower values of motility and kinetic parameters. Regarding sea bass and sole, motility and kinetic parameters were not affected by seawater pH (6.5 to 9.5). In the case of seabream, the motility was only reduced at the most basic pH (9.5). On the other hand, we observed that the beating cross frequency in eel spermatozoa was lower at 23 °C than at 4 °C. Elevated temperature (22 ºC) reduced the sperm motility in sea bass, especially when combined with low pH. In the case of the sole, low temperatures (4 ºC) at low pH (7.8) seemed to decrease motility. In the seabream, there were no differences in the motility of the sperm at natural (16 ºC) or at higher temperatures (22 ºC). The differences found among these species, being evolutive and ecologically diverse, could reflect different activation mechanisms of the spermatozoa motility and thus be affected by the rapid climate change.

Acknowledgements. This study was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (THINKINAZUL/2021/012; THINKINAZUL/2021/024; THINKINAZUL/2021/042). WAG has a Margarita Salas postdoctoral contract (RD 289/2021. UAB) by the Spanish Ministry of Universities. TSF and MPS have fellowships from Brazilian institutions: CNPq (200285/2021-1), and CAPES (Finance code 001), respectively.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

EFFECT OF HIGH REARING TEMPERATURE ON GROWTH AND GONAD MATURATION OF JUVENILE MALE EUROPEAN SEA BASS (Dicentrarchus labrax)

Sarih S[1,2*], Zapater C[1], Benítez A[3], Gómez A[1], Felip A[1].

[1]Fish Reproductive Physiology Group, Department of Fish Physiology and Biotechnology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellón, Spain. [2]Aquaculture Research Group, IECOAQUA, University of Las Palmas de Gran Canaria (ULPGC), Crta. Taliarte s/n, 35214 Telde, Las Palmas, Spain. [3]Catholic University of Valencia, C. Guillem de Castro, 46001, Valencia, Spain. *E-mail: sarih.samira@gmail.com

The rapid climate change and rising ocean temperatures cause negative impacts on fishes, leading to alterations of metabolic processes, growth and reproduction. The exposure of adult fish to warm temperatures is known to impair reproduction, although the long-term reproductive impact during early life is not well clarified. This study aimed to evaluate the effects of warm rearing temperatures on growth and gonad maturation in juvenile male European sea bass during their first year of age. Juvenile sea bass (14.0 ± 4.1 g initial body weight) were established in two experimental groups in triplicate: i) natural seawater temperature (control group, 40°08′15″ N; 0°10′12″ E) and ii) 3-4 ºC above the temperature of the control group (temperature group) for 10 months (July-April). Growth, biometric parameters, testicular growth and development and circulating plasma levels of follicle-stimulating hormone (Fsh), testosterone (T) and 11-ketotestosterone (11-KT) were analyzed. The elevation of rearing temperature reduced the long-term growth performance of juvenile male European sea bass during their first year of life. All fish started gametogenesis, but fish in the temperature group showed a delay in the progression towards advanced testicular stages. In addition, the highest proportion of precociously spermiating males was observed in the control group in February with 78% in comparison to 23% in the temperature group. Of note, spermiation lasted longer in the control group. Also, males in the temperature group exhibited a decrease of plasma Fsh, whereas the levels of T and 11-KT remained unchanged in both experimental groups. In conclusion, rising water temperature affects the growth and gonadal development of the European sea bass males during their first year of life by affecting the reproductive axis at multiple levels.

Acknowledgements. This research was supported by MICIN /NextGenerationEU (PRTR-C17.I1) /Generalitat Valenciana (THINKINAZUL/2021/042). S.Sarih was supported by a research contract from Margarita Salas program from ULPGC.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

BROWN TROUT PRIMARY HEPATOCYTE SPHEROIDS – CHARACTERIZATION AND APPLICATIONS IN ENDOCRINOLOGY AND BEYOND

Madureira TV[1,2]*, Alves R[1,2], Lopes C[1,2], Rašković B[3], Rocha E[1 2].

[1]Team of Animal Morphology and Toxicology, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto (U.Porto), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. [2]Laboratory of Histology and Embryology, Department of Microscopy, ICBAS — School of Medicine and Biomedical Sciences, University of Porto (U.Porto), Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. [3]Institute of Animal Sciences, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia. *Email: tvmadureira@icbas.up.pt.

Three-dimensional (3D) culture models of mammalian hepatocytes have been shown to replicate in vivo liver features accurately. The use of these 3D models avoids the constraints of an in vivo assay while sustaining the morphology and functionality of the hepatocytes throughout several days in culture. The feasibility of generating hepatic spheroids from other species, including fish, seems promising but has received less attention than in mammal models.

Primary brown trout hepatocyte spheroids were first obtained by our research group from juvenile fish and cultured for over 30 days under orbital shaking. Optimization assays showed that these spheroids achieved biometric, morphological and basal gene expression stability from day 12 to day 20. This multiparametric characterization allowed the selection of a temporal window of culture days, which is being explored in several assays. The 3D primary hepatocyte spheroids were exposed to estrogens (e.g., 17α-ethinylestradiol) at different concentrations. The effect of increasing temperature was also evaluated in this model by maintaining cultures at 18°C and 21°C. Data collected include microscopy analysis, target gene expression of estrogenic and lipid pathways, and cellular biochemistry. The outputs indicate that hepatic spheroids are metabolically active and respond to different hormonal and temperature stimuli. The 3D model developed from primary brown trout hepatocytes seems to be a viable alternative for studying hepatic disruptions caused by (at least) estrogenic compounds and varied temperatures.

Acknowledgements. EU's Horizon 2020 research and innovation programme (Marie Sklodowska-Curie grant agreement No. 101038087); project ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); Strategic funding UIDB/04423/2020 and UIDP/04423/2020, through FCT and ERDF to CIIMAR/CIMAR.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TEMPERATURE AND ENDOCRINE-DISRUPTORS EFFECTS IN THE TROUT LIVER RTL-W1 CELL LINE

Vilaça M[1,2], Rocha E[1,2], Seabra R[1,2], Lopes C[1,2]*.

[1]Laboratory of Histology and Embryology, Department of Microscopy, ICBAS – School of Medicine and Biomedical Sciences, University of Porto (U.Porto), Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. [1]Team of Animal Morphology and Toxicology, [2]CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto (U.Porto), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. *Email: cclopes@icbas.up.pt.

The water temperature increase due to global warming affects the metabolic processes of ectothermic animals, like fish. Aquatic ecosystems are also disturbed by ubiquitous pollutants, including endocrine-disrupting compounds (EDCs), such as 17-α ethinylestradiol (EE2) and levonorgestrel (LNG). Since warming may alter how organisms metabolise and respond to xenobiotics and vice versa, exploring the combined effects of temperature and EDCs is critical. Fish liver cell lines may be a valuable model to study these mixed effects. We aimed to test if the trout liver RTL-W1 cell line can be used as a model to study temperature influences on EDC's effects. We thus exposed RTL-W1 cells (72 h), at 18 ºC and 21 ºC, to EE2, LNG, a mixture of both hormones (MIX), at 10 µM. After the exposures, cell viability was assessed using trypan blue exclusion dye. Gene expression of targets related to metabolization, detoxification, estrogenic and lipid metabolism was analysed by RT-qPCR. Neither exposure conditions nor temperature affected cell viability. The two-way ANOVA revealed that GST expression was higher at 21ºC than at 18ºC but not influenced by EDC's exposure. MRP2 and FAS mRNA levels were higher in the MIX compared with control. CAT expression was increased by LNG and CYP3A27 by LNG and MIX conditions, compared with control. VtgA, UGT, CYP1A and HSP70b expressions were not influenced by temperature or the tested EDCs. The results are not final, but suggest interferences of estrogens and progestins in several signalling pathways that deserve to be explored. The RTL-W1 cell line appears to have potential to study the interaction between temperature and EDCs.

Acknowledgements. ATLANTIDA - Platform for monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), by NORTE 2020, under PORTUGAL 2020, through the ERDF; FCT strategic funding UIDB/04423/2020 and UIDP/04423/2020, to CIIMAR/CIMAR.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

APPLICATION OF BROWN TROUT PRIMARY HEPATOCYTES SPHEROIDS TO TEST 17A-ETHINYLESTRADIOL (EE2) EFFECTS

Alves R[1,2]*, Lopes C[1,2], Rocha E[1,2], Madureira TV[1,2].

[1]Team of Animal Morphology and Toxicology, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto (U.Porto), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. [2]Laboratory of Histology and Embryology, Department of Microscopy, ICBAS — School of Medicine and Biomedical Sciences, University of Porto (U.Porto), Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. *Email: rodrigo.r.f.alves@hotmail.com

The metabolism, bioaccumulation, and biotransformation pathways have been successfully studied in mammals using three-dimensional (3D) hepatocyte cultures (spheroids). Compared to 2D cultures, the 3D models allow sustaining hepatocytes over larger periods, with morphology and function closer to in vivo systems. In fish, the application of 3D cultures have been increasing, but is still limited. Here, we used a spheroid model derived from juvenile brown trout (*Salmo trutta*) primary hepatocytes, as previously developed by our research group. The aim was to test the competency of hepatocyte spheroids to respond to estrogenic stimulation. For this, spheroids were exposed to a classic estrogenic compound (EE2), at distinct concentrations (1 ng/L, 10 ng/L, 50 ng/L and 100 ng/L), from the 12th day to 18th day post-isolation. Exposure occurred in 6-well plates where the cells were plated initially, and medium changes were performed on alternate days. The spheroids were evaluated in terms of cell viability (lactate dehydrogenase – LDH and resazurin assays), biometry (equivalent diameter, area, and sphericity) and RT-qPCR analyses of several estrogenic-responsive genes: vitellogenin A (VtgA) and estrogenic receptors α/β (ERα and ERβ). No differences were found in the viability and biometry of spheroids between exposure groups. For the highest concentrations, there was up-regulation of specific targeted genes, namely VtgA. The data sustain that the tested spheroids are an in vitro model able to evaluate endocrine-disruption on hepatocytes by estrogenic compounds. Studies are needed to conclude if they better mimic in vivo responses.

Acknowledgements. Project ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145- FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through FCT and ERDF to CIIMAR/CIMAR.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CHARACTERISATION OF THICKLIP GREY MULLET AND POLAR COD PPARα AGONISTS BY USING GENE REPORTER ASSAYS

Vinacua S[1], Alonso A[2], Colliar L[3], Vieweg I[3], Leaver M[3], Cajaraville MP[1,2], Nahrgang J[4], Cancio I[1,2], Bilbao E[1,2]*.

[1]*CBET+ Research Group, PiE-UPV/EHU, University of the Basque Country (UPV/EHU), Areatza Hiribidea s/n, 48620 Plentzia, Basque Country, Spain. [2]CBET+ Research Group, Dept. Zoology & Animal Cell Biology, Science & Technology Faculty, UPV/EHU. [3]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, FK9 4LA Stirling, UK. [4]Department of Arctic & Marine Biology, Faculty of Biosciences, Fisheries & Economics, UiT the Arctic University of Norway, N-9037, Tromsø, Norway. *Email: eider.bilbao@ehu.eus

Organic compounds, including petroleum derived hydrocarbons have been reported to alter lipid metabolism and cause the proliferation of peroxisomes in different tissues on marine organisms. Therefore, peroxisome proliferation is usually studied as a biomarker of exposure to organic xenobiotics in order to assess the health of marine environments. This cellular response is mediated by the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα), whose ligand binding domain (LBD) binds substrates that are able to activate the transcription of target lipid metabolism genes. In the present study, endogenous and exogenous agonists potentially able to activate PPARαLBD were tested in two teleost sentinel species inhabiting different ecosystems: the estuarine thicklip grey mullet *Chelon labrosus* and the polar cod *Boreogadus saida*. Three luciferase gene reporter assays were created utilizing the PPARαaLBD of mullets and the PPARαaLBD and PPARαbLBD of polar cod. These reporters where then used to study the activity of putative PPARα agonists in transactivation assays. The mullet reporter gene was activated by the prototypical PPARα ligand Wy14643 and by different kinds of short-, medium- and long-chain fatty acids. Interestingly, polar cod PPARαbLBD was activated at low concentrations of the same ligands, while PPARαaLBD was not activated by any, suggesting polar cod PPARαbLBD is the ligand dependent form. Differences in PPARαLBD sequences lead to different 3D structures, which could help to explain results in the transactivation assays in both species. However, further research will help to characterize exogenous agonists, including xenobiotics such as perfluorinated compounds and PAHs, for each fish PPARαLBD in order to use mullet and polar cod luciferase gene reporter assays to assess the presence of peroxisome proliferators in temperate estuarine and polar environments.

Acknowledgements. Work funded by the Basque Government (Grant to consolidated research groups IT1743-22 and REPORTER SAIOTEK SA-2010/00038) & Research Council of Norway (POLARISATION REF: 214184).

SESSION 4.
GROWTH
AND
DEVELOPMENT

PLENARY LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

MORPHOLOGICAL ADAPTATIONS AND GENE EXPRESSION PATTERNS IN FLATFISH EYE MIGRATION: INSIGHTS INTO VISUAL SYSTEM DEVELOPMENT AND FUNCTION

Guerrero-Peña L[1]*, Suarez-Bregua P[1], Van Dyck A[2], Sánchez-Ruiloba L[1], Méndez-Martínez L[1], Tur R[3], García-Fernández P[3], Moons L[2], Tena JJ[4], Rotllant P[1].

[1]*Aquatic Biotechnology Lab., Institute of Marine Research, Spanish National Research Council (IIM-CSIC), 36208, Vigo, Spain. [2] Neural Circuit Development and Regeneration Research Group, Department of Biology, KU, Leuven, Naamestraat 61, box 2464, 3000 Leuven, Belgium. [3] Nueva Pescanova Biomarine Center, S.L., 36980, O Grove, Spain. [4] Centro Andaluz de Biología del Desarrollo (CABD), CSIC-Universidad Pablo de Olavide, 41013 Sevilla, Spain. *Email: lguerrero@iim.csic.es

Predatory fish heavily rely on their visual capabilities to accurately detect and track theis prey. As a result, some species have developed unique adaptations in their morphology and visual system to facilitate ambush or shelter behavior. Flatfish, undergo a remarkable morphological transformation during a complex metamorphic process. In flatfish, one eye migrates to opposite side of the body, enabling them to live as benthic juveniles. Alongside this adaptation to varying light conditions, the eyes undergo several morphological asymmetries to accommodate the migrating eye to its future ocular side. Previous studies have also observed asymmetry in the length of the optic nerve, with the non-migrating eye having a shorter optic nerve. Although it has been observed that the migrating eye of flatfish has a smaller optic tectum during metamorphosis, it remains unclear whether this eye is less functional than the non-migrating eye. In our study, we conducted a transcriptome analysis of eyes and surrounding tissue at three crucial stages of turbot metamorphosis (pre-metamorphic, climax and post-metamorphic stages). We discovered an intriguing and unique expression pattern in the ependymin (*epd*) gene. Our data revelead a peak of *epd* expression during the climax of metamorphosis in the migrating eye, while there were no changes in the temporal expression pattern in the non-migrating eye. EPD is a glycoprotein found in brain of teleosts and is involved in long-term memory, neural plasticity and refinement of connections in regenerating optic nerve. To further investigate the potential role of *epd* in eye migration, we created an *epd* knockout mutant fish (*epd* $^{K.O.}$). We examined the axonal regrowth and dendritic remodeling after optic nerve injury in *epd* $^{K.O.}$ fish, comparing studying the differences in regeneration between wildtype and mutant fish and exploring the possible correlation with optic nerve asymmetry in flatfish.

Acknowledgements. Project funding: AGL2017-89648P, PID2021-1265110B-100.

ORAL COMMUNICATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

GROWTH AND PHYSIOLOGICAL PERFORMANCE OF FEMALE EUROPEAN SEA BASS WITH DIFFERENT BODY SIZE AND GONADAL DEVELOPMENT: COMPARISON OF RNA TRANSCRIPTS FROM LIVER TISSUE

Sempere L[1]*, Mukiibi R[2], Ibáñez S[1], Molés G[1], Calduch-Giner J[1], Pérez-Sánchez J[1], Viñas A[3], Martínez P[3], Robledo D[2], Felip A[1].

[1]*Aquaculture Institute of Torre de la Sal (IATS), CSIC, Ribera de Cabanes s/n 12595, Castellón, Spain. [2]The Roslin Institute, Easter Bush Campus, Midlothian EH25 9RG, Edinburgh, United Kingdom. [3]Departamento de Zooloxía, Xenética e Antropoloxía Física, Universidade de Santiago de Compostela, Spain. *Email: laurasb@iats.csic.es

Female European sea bass (Dicentrarchus labrax) display faster growth rates than males, therefore a monoculture of this sex could be beneficial for production. In captivity, 2-yr-old females weighing around 500 g show an early onset puberty with oocytes in late vitellogenesis stage. However, there is no evidence that these females are spawning fish. Nevertheless, there is substantial variation in body size and gonad growth between females with an early and late onset puberty, and this phenomenon should be given consideration as it might reduce harvest efficiency. In our study, we identified four female phenotypic categories according to their body size; Small (S) and Large (L), as well as gonadal development; Immature (I) and Advanced (A) which we designated as SI, SA, LI and LA at 2-yr-old. Animals were obtained from three lots of fertilized eggs; they were individually tagged, and periodically measured for body weight and length. Blood samples were collected to profile levels of the plasma follicle-stimulating hormone (Fsh), 17β-estradiol (E2), vitellogenin (Vtg) and the insulin-like growth factor-1 (Igf-1). Body indexes, gonadal tissue for histological analysis and liver tissue for transcriptomic analysis were obtained. Results showed that LI and LA females were 2.17 times heavier than SI and SA fish, while SA and LA females had a higher gonadosomatic index in comparison to that of SI and LI fish (3.1 vs 0.6%, respectively). SA and LA females showed higher plasma levels of Fsh, E2 and Vtg than SI and LI. Circulating levels of Igf-1 were similar in all groups. Liver transcriptomic analysis identified 13 differentially expressed genes between the comparisons of the four phenotypic categories. Pathways involved in signal transduction, lipid metabolism and endocrine system are being studied.

Acknowledgements. Project funded from MCIN (AGL2016-75400, PID2019-109548RB-I00). CESGA (http://www.cesga.es) provided computing facilities. L.S. supported by a FPI fellowship from MCIN (Spain).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

DNA METHYLATION DURING EARLY DEVELOPMENT IN DIPLOID AND TRIPLOID EUROPEAN SEA BASS

Beato S[1]*, Sánchez-Baizán N[1], Felip A[2], Piferrer F[1].

[1]*Group of Biology of Reproduction (GBR), Department of Renewable Marine Resources, Institut de Ciències del Mar (ICM), Consejo Superior de Investigaciones Cientificas (CSIC), Passeig Marítim, Barcelona, Spain.
[2]Group of Fish Reproductive Physiology, Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (IATS), Consejo Superior de Investigaciones Cientificas (CSIC), Ribera de Cabanes, Castellón, Spain. *Email: silviabeato@icm.csic.es

The induction of triploidy is used in the aquaculture of some fish and mollusks to enable maximizing growth and mitigate the problems associated with sexual maturation. The main consequence is the sterility of the triploids, particularly of females. Induced triploidy can be achieved by retaining the second polar body through physical shocks. This has a significant impact on the survival and development of embryos. A research revealed disrupted gene expression in allotriploid hybrid grouper during the middle gastrula and crystal (hatching) stages. However, our understanding on the underlying epigenetic mechanisms remains limited, and the existing literature presents conflicting results. Thus, no differences were observed in brown trout (*Salmo trutta*). In contrast, in triploid oysters (*Crassostrea gigas*), hypo- and hypermethylated regions were observed in infertile and fertile oysters, respectively. A dosage compensation by DNA methylation allows resembling diploid gene expression levels, in allotriploid cyprinids. Therefore, the objective of our study was to investigate the effects of triploidization on DNA methylation patterns, during embryo development of triploid European sea bass (*Dicentrarchus labrax*). Triploidization was induced by a cold shock. Two stages of development (90% epiboly, 30 hpf and hatching, 92.5 hpf) were analyzed. A mean of 30,680 differentially methylated cytosines (DMCs) were identified as either hyper- or hypomethylated in both stages, when compared to the corresponding diploids. DMCs were distributed along the entire genome, and were mostly concentrated in intergenic regions and CpG islands. Two genes, *phrf1* and *acss1,* identified as differentially expressed in hybrid grouper, had DMCs close to their transcription starting sites in the sea bass. GO term and KEGG enrichment analysis will be reported to clarify if the higher initial mortality is only a direct consequence of the shock, or also have an epigenetic basis.

Acknowledgements. Research funded by Spanish Ministry of Science and Innovation 'Epipure' (PID2019-108888RB-I00) grant to FP.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SPECIES-SPECIFIC RESPONSE OF TWO MARINE BIVALVES TO OCEAN ACIDIFICATION

Peng MX[1]*, Sorigué P[1], Cardoso JCR[1]*, Power DM[1,2,3]*.

[1]Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. [2]International Research Center for Marine Biosciences, Ministry of Science and Technology, Shanghai Ocean University, Shanghai, China. [3]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China.
*Email: 66019@ualg.pt

Bivalves are a large and highly diverse group of sessile filter-feeding aquatic invertebrates that are characterized by their protective calcareous biomineralized shell. Currently, bivalve populations are under threat from Ocean acidification (OA, due to increased atmospheric CO2), which impacts shell emergence, maintenance and composition. However, the impact of decreased pH on bivalve shells is likely to vary considerably across species and life stages but few comparative studies exist. To explore how bivalves with different shell compositions and morphologies respond to OA we challenged two important marine aquaculture bivalves, the mussel *M. galloprovincialis* (hard regular shell) and the Pacific oyster *C. gigas* (softer foliated shell) to acidified seawater (pH 7.8 as predicted for 2100 by IPCC) for 60 days and compared their response. OA treatment impeded shell growth in both species and animals exposed to OA were significantly smaller ($p < 0.001$) in shell-length/shell area compared to the control and the periostracum (outer-shell) and the nacreous layer (inner-shell) of the mussel was modified. Transcriptomes of the shell secreting mantle of both species revealed they respond differently to OA and the number of differentially expressed genes (DEG) in the mussel (787 DEG) mantle edge was higher than in oyster (103 DEGs) and only a small proportion of overlapping homologous genes were found. DEGs covered a wide range of functions and GPCRs linked to regulatory processes were identified. DEG GO enrichment analysis revealed that they were mainly distributed in the categories Biological regulation, Cellular process, Developmental process, Metabolic process and Response to stimulus but in the mussel a higher proportion were associated with Immune system process. Our data confirm that bivalves with different shell types respond differently to OA and that the mussel is more responsive than the oyster and that many of the activated mussel genes were related to the immunity.

Acknowledgements. This study received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020. MP was supported by a PhD scholarship from Shanghai Ocean University (China)

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

TOXICOLOGICAL RISKS OF METFORMIN FOR AQUATIC ECOSYSTEMS: A GENERATIONAL STUDY WITH ZEBRAFISH USING ENVIRONMENTALLY RELEVANT CONCENTRATIONS OF METFORMIN

Barros S[1,2], Ribeiro M[1,3], Pinheiro M[1,3], Alves N[1,3], Morais H[1,3], Montes R[4], Rodil R[4], Quintana JB[4], Coimbra AM[2,5], Santos MM*[1,3], Neuparth T[1].

[1]*CIIMAR—Interdisciplinary Centre of Marine and Environmental Research, Endocrine Disruptors and Emerging Contaminants Group, University of Porto, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal. [2] CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, Pavilhão 2, 5000-801 Vila Real, Portugal. [3] FCUP - Department of Biology, Faculty of Sciences, University of Porto (U. Porto), Rua do Campo Alegre s/n, 4169-007 Porto, Portugal. [4] Department of Analytical Chemistry, Nutrition and Food Sciences, IAQBUS - Institute of Research on Chemical and Biological Analysis, Universidade de Santiago de Compostela, Constantino Candeira S/N, 15782 Santiago de Compostela, Spain. [5] Inov4Agro –Institute for Innovation, Capacity Building and Sustainability of Agri-food Production, Portugal. *Email: miguel.santos@fc.up.pt

Metformin (MET), an antidiabetic pharmaceutical widely consumed worldwide, has been detected in surface waters at concentrations ranging from ng/L to low µg/L. However, the long-term effects of environmentally relevant MET concentrations on non-target organisms remain poorly understood. To address this knowledge gap, the present study aimed to investigate the generational effects of MET on the model organism *Danio rerio* (up to 9 mpf), using concentrations ranging from 390 to 14 423 ng/L. The study also investigated the impacts of the parental exposure (F0) on the early life stages of the nonexposed F1 generation (intergenerational effects). A comprehensive approach was used by integrating several apical endpoints, including embryonic development, survival, growth, and reproduction, with molecular (qRT-PCR and RNA-seq) and biochemical (cholesterol and triglycerides content and mitochondrial complex I activity) analyses to gain further insights into the underlying MET's mode of action. Adverse effects were observed across multiple biological functions, including lipid/carbohydrate metabolism, survival, growth, and reproduction. Moreover, the molecular analyses conducted provided compelling evidence that MET acts as an endocrine-disrupting chemical (EDC) by interfering with the biosynthesis of steroid hormones, even at concentrations as low as 390 ng/L. These findings indicate that current predicted no-effect concentrations (PNEC) and environmental quality standards (EQS) recently established for MET need to be revised, as they seriously underestimate the long-term toxicity of this pharmaceutical.

Acknowledgements. This study was developed under the project - TRANSEPIC − Exploring Transgenerational Epigenetic Inheritance: New Methods and Strategies to Improve Environmental Hazard and Risk Assessment of Key Contaminants of Emerging Concern (CECs) [Reference: 2022.02922.PTDC], financed by the Portuguese Foundation for Science and Technology (FCT). S. Barros and T. Neuparth acknowledge FCT for their grants PD/BD/143090/2018 and Individual Call to Scientific Employment Stimulus 2022 (2022.02925.CEECIND), respectively.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

EMBRYO BIOASSAYS FOR TOXICITY TESTING: DEVELOPMENT OF LYMNAEA STAGNALIS EMBRYO-TESTS FOR THE HAZARD ASSESSMENT OF CONTAMINANTS OF EMERGING CONCERN

Capela R[1, 2, 3]*, Castro LFC[1, 2], Santos MM[1,2], Garric J[3].

[1]CIMAR/CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. [2] FCUP – Faculty of Sciences of the University of Porto; Rua do Campo Alegre s/n, 4169-007 Porto, Portugal. [3] INRAE - National Research Institute for Agriculture, Food and the Environment - Centre de Lyon-Villeurbanne; 5 rue de la Doua, CS20244, 69625 Villeurbanne Cedex, Lyon-Villeurbanne, France.*Email: ricardocapela.c@gmail.com

The validation of high-throughput toxicity tests using invertebrates is crucial for improving hazard assessment of new chemicals and expanding test guidelines across different organism taxa. This approach aligns with new legal trends promoting the 3R policy (reduction, refinement, and replacement of animal experiments) and emphasizes the potential of embryo-tests for high-throughput toxicity screening. Existing test guidelines primarily focus on vertebrates and arthropods, limiting the extrapolation of hazard assessments to the ecosystem level. To address this limitation, the study aims to validate an embryo-test with *Lymnaea stagnalis*, an important test animal model identified by the OECD. While a reproductive test has been standardized, validating an embryo toxicity test is essential to cover the sensitive period of embryogenesis. The study follows OECD guidelines and previous works on mollusk life-cycle toxicity testing. Criteria such as mortality rates, abnormality rates, development, growth, hatching rates, and testing media suitability are validated. Cadmium was chosen as the positive test substance due to available ecotoxicity data on several invertebrates. The results show the higher sensitivity of *L. stagnalis* embryos to cadmium than adult stages, when using the developed embryo-test protocol, and a dose-response correlation, with EC10 and EC50 values of 13.57µg/L and 21.84 µg/L at 240 hours, respectively. The results also allow to propose values range for the measured toxicity criteria. In conclusion, the study highlights the high-throughput testing capability and sensitivity of the *L. stagnalis* embryo test. The findings contribute to future standardization of the assay and emphasize the importance of validating invertebrate-based tests for comprehensive hazard assessment. The study advocates for broader inclusion of taxa in test guidelines to ensure ecosystem-level hazard assessment.

Acknowledgements. This work was supported by the projects Atlantida [NORTE-01-0145-FEDER-000040]. By the Portuguese Foundation for Science and Technology (FCT) under the Grant SFRH/BD/112483/2015 and the "Pessoa" Program (2018-2019) CIIMAR/IRSTEA. Miguel Santos acknowledge financing through the project NOR-WATER (0725), financed by "Programa de Cooperação Interreg Portugal/Espanha", (POCTEP) 2014-2020.

POSTER PRESENTATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

SEEKING FOR THE INVOLVEMENT OF THE IGF SYSTEM IN THE ONSET OF EARLY SEXUAL MATURATION IN MALE EUROPEAN SEA BASS, Dicentrarchus labrax L.

Sempere L, Ibáñez S, Molés G, Pérez-Sánchez J, Felip A*.

Institute of Aquaculture Torre de la Sal (IATS), CSIC, Ribera de Cabanes s/n 12595, Castellón, Spain.
*Email: afelip@iats.csic.es

Male European sea bass usually mature at 2-yr-old, however, a high proportion of fish mature earlier during the first year of age. Precocious males are heavier than their non-precocious counterparts at 1-yr-old, but their growth is reduced during their second year of life. In this study, we analyzed the relationship among some key reproductive indicators and the elements of the Igf system which might play a critical role in the interaction between the somatotropic and gonadotropic systems. Fish were obtained and maintained under natural conditions at IATS facilities. Growth, body indexes and gonadal development were evaluated during the first year of age. The stage of testicle development was histologically assessed, with stages IV and V designated as indicators of maturity. Blood samples were collected and plasma levels of the follicle-stimulating hormone (Fsh), sex steroids and the insulin-like growth factor-1 (Igf-1) were measured. The principal component analysis (PCA) of all the factors measured showed that >73% of total variance was explained by the first two components. The PCA showed that weight, Igf-1 and Fsh were key factors influencing early sexual maturation, whereas plasma estradiol levels were higher in immature males. Changes in gonad gene expression of igf-1, -2 and -3 were analyzed by qRT-PCR as well as other key genes involved in gonad growth. Results showed that igf genes showed stage specific expression during gonad development with high expression levels from immature to gonadal mid recrudescence stage. These findings suggest that igf system might play a critical role, at least, in the progress of cellular proliferation and/or meiosis during early stages of spermatogenesis and influence the early onset of puberty in this teleost marine fish.

Acknowledgements. Project funded from MCIN (AGL2016-75400, PID2019-109548RB-I00). LS supported by a FPI fellowship from MCIN (Spain).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ROLE OF *miR-133* FAMILY IN THE DEVELOPMENT OF GILTHEAD SEABREAM (*Sparus aurata*) FAST SKELETAL MUSCLE

García-Pérez I, Ginestà J, Perelló-Amorós M, Capilla E*, Navarro I, Blasco J, Gutiérrez J, Garcia de la serrana D.

Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain. *Email: ecapilla@ub.edu

MicroRNAs (miRNAs) are a group of small non-coding RNAs that play an important role in the regulation of gene transcription by binding to target mRNAs and triggering their translational repression or promoting their degradation. Currently, there is limited knowledge about the role of miRNAs in the regulation of skeletal muscle growth in fish. Hence, this study aimed to explore the role of *miR-133* family (*miR-133a-1*, *miR-133a-2* and *miR-133b*) in muscle development and growth of gilthead seabream (*Sparus aurata*). Bioinformatic approaches were used to analyze the genomic localization and to predict the target mRNAs of the miRNAs. To examine the degree of conservation of *miR-133* family among species, a phylogenetic tree was constructed. Tissue distribution of these miRNAs was analyzed and their transcription in gilthead seabream white muscle under different physiological conditions. All three miRNAs showed higher expression in both fast and slow skeletal muscles compared to other tissues, and *miR-133a-1* and *miR-133a-2* had higher transcription in fast muscle, whereas *miR-133b* transcription was higher in slow muscle. Interestingly, *miR-133a-1* and *miR-133a-2* expression was similar in the three ontogenetic stages (fingerlings, juveniles, and adults) for fast muscle, while *miR-133b* showed significantly higher expression in fingerlings, suggesting an important contribution of this miRNA in the regulation of muscle development and growth in early stages. Under fasting conditions, all three miRNAs showed similar transcription levels in fast muscle, whereas *miR-133b* increased after 2 hours of refeeding, indicating that it might be involved in the rapid response to nutritional inputs. Overall, these results suggest that *miR-133* family members' transcription is differentially regulated, and that *miR-133b* may have a distinct role in myogenic regulation than *miR-133a-1* and *miR-133a-2* in gilthead seabream.

Acknowledgements. Study supported by the project RTI2018-100757-B-I00 from the MCIN/AEI. I.G.-P. and M.P.-A. were supported by predoctoral fellowships PRE2019-089578 and BES-2016-078697, respectively.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CHARACTERIZATION OF GILTHEAD SEA BREAM (*Sparus aurata*) OSTEOBLASTS SECRETOME

Rodríguez I*, Thomas-García J, Martín-Velaz L, Garcia de la serrana D, Gutiérrez J, Navarro I, Capilla E.

Department of Cell Biology, Physiology and Immunology, University of Barcelona, Barcelona 08028, Spain.
*Email: Inmaculada.rodriguez@ub.edu

Bone can act as an endocrine organ through the secretion of proteins called 'osteokines'. In mammals, different osteokines have been identified such as bone-derived fibroblast growth factor 23, osteocalcin and lipocalin-2, which are involved in phosphate homeostasis and energy metabolism among other functions; however, in fish, osteokines remain unexplored. The aim of this study was to identify potential osteokines secreted by gilthead sea bream (*Sparus aurata*) cultured osteoblasts and, to analyse *in vitro* their influence in adipocytes. To this end, gilthead sea bream bone-derived cells were cultured in either growth or osteogenic medium for 15, 20 or 40 days, and further incubated for 24 h in a serum- and red phenol-free medium to obtain the bone-conditioned medium (BCM). To characterize the secretome, 30 ml of BCM previously centrifuged to remove cells and debris, were precipitated using trichloroacetic acid and resuspended in urea 6 M to obtain the protein fraction. A 7.5% SDS-PAGE was run and stained with colloidal Coomassie to visualize the proteins. Profiles and bands intensities varied between samples from the different media and culture day suggesting the presence of secreted proteins. Additionally, a proteome specific analysis is being performed using LC-MSMS mass spectrometry. To explore osteokines function, BCM from 20 days developed bone-derived cells grown in osteogenic conditions was collected and stored at -80ºC. Then, primary cultured adipocytes obtained from the perivisceral adipose tissue of gilthead sea bream were incubated, starting from day 0 or day 8, with fresh medium replaced with 20% or 40% of BCM. On day 11, cell viability and lipid accumulation by means of colorimetric assays (MTT and Oil Red O staining) were determined. The values obtained tended to increase with the percentage of replacement, suggesting a possible endocrine/paracrine effect of the bone secretome on gilthead sea bream adipocytes.

Acknowledgements. Supported by MICIN (PID2020-116172RB-I00).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

EFFECTS OF CO_2 ON THE PROLIFERATION AND MINERALIZATION OF GILTHEAD SEA BREAM (*Sparus aurata*) CULTURED OSTEOBLASTS

Rodríguez I*, Martín-Velaz L, Thomas-García J, Montblanch M, Garcia de la serrana D, Gutiérrez J, Navarro I, Capilla E.

Department of Cell Biology, Physiology and Immunology, University of Barcelona, Barcelona 08028, Spain.
*Email: Inmaculada.rodriguez@ub.edu

Bone turnover is directly correlated with pH, since resorption by osteoclasts occurs at acidic conditions, while in alkaline pH, collagen synthesis and bone formation is stimulated by osteoblasts. Traditionally, using cell culture protocols from mammals, the pH of buffers and media is stabilized at 7.4 according to the blood pH; however, the local pH of other tissues may differ significantly; besides fish blood pH might be slightly higher (i.e., 7.6-7.8). Thus, the aim of this study is to determine how pH might modify gilthead sea bream (*Sparus aurata*) osteoblasts in culture. To this end, vertebra bone-derived cells from this species were incubated during 30 days in either growth medium or osteogenic medium at 23ºC and, three different CO_2 levels: 0%, 1.5% and 2.5%. Data showed that at CO_2 concentrations of 2.5% and 1.5%, the pH remained close to the physiological level (7.33±0.03 and 7.54±0.07, respectively), especially at 1.5%. Contrarily, under the 0% CO_2 condition, there was an alkalinization of the medium (8.02±0.12). Cell proliferation was assessed every 5 days using the MTT assay and mineralization by means of Alizarin Red staining. At 2.5% and 1.5% of CO_2, significant increases in proliferation from day 5 and in mineralization from day 10 were found, regardless of the incubation media (growth medium or osteogenic medium). At day 30, significant higher levels of both, proliferation and mineralization were detected in cells incubated with osteogenic medium at 0% CO_2 compared with the other culture conditions. These results indicated that alkalinization appears to be beneficial for osteoblasts development, although these conditions resulted in non-physiological pH values. Overall, this study provides information about how the CO_2 / pH conditions can affect bone growth and mineralization, and help to dilucidate if an elevated pH is better for the cultivation of fish bone cells.

Acknowledgements. Supported by MICINN (PID2020-116172RB-I00).

SESSION 5.
FOOD INTAKE
AND
METABOLISM

PLENARY LECTURE

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ESTROGENIC AND STATIN IMPACTS IN LIPID PATHWAYS OF BROWN TROUT

Lourenço T[1,2]*, Vieira Madureira T[1,2], Rocha MJ[1,2], Rocha E[1,2].

[1]Laboratory of Histology and Embryology, Department of Microscopy, ICBAS – School of Medicine and Biomedical Sciences, University of Porto (U.Porto), Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. [1]Team of Animal Morphology and Toxicology, [2]CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto (U.Porto), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. *Email: up201707353@up.pt

Hypolipidemic drugs and synthetic estrogenic compounds are prevalent environmental pollutants capable of causing dyslipidemia in fish. Within this context, this study investigated the effects of 17α-ethinylestradiol (EE2; a synthetic estrogen), atorvastatin (ATV; a hypolipidemic drug), and a combination of both (MIX) in juvenile brown trout. Control was a saline solution, and solvent control was the saline solution fortified with ethanol and DMSO. Fish (n=10/condition) received intramuscular injections twice a week for two weeks. Endpoints included biometry, blood lipid biochemistry, hepatic lipid droplets quantification, and liver mRNA expression. Liver weight and hepatosomatic index (HSI) increased in the EE2 and MIX groups. Triglycerides were higher in the EE2 animals and lower in the ATV. ATV also reduced cholesterol and LDL. HDL levels diminished across all groups. The deposition of lipid droplets in the cytoplasm of hepatocytes was higher in the EE2 and MIX groups, and all exposed groups showed heterogeneity in the size and spatial distribution of the droplets. Estrogen receptor α (ERα) and vitellogenin A (VtgA) mRNA levels were upregulated by EE2 and MIX. Likewise, the classic statin target 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoAR) was upregulated by EE2 and ATV. Acyl-CoA long chain synthetase 1 (Acsl1) was upregulated by EE2, whilst fatty acid binding protein 1 (Fabp1) showed an inverse pattern. EE2 and MIX caused a downregulation of apolipoprotein AI (ApoAI) whereas ATV upregulated it. ATV and MIX reduced peroxisome proliferator-activated receptor γ (PPARγ) mRNA levels. All three treatments decreased the gene expression of acetyl Co-A oxidase 1 - 3I (Acox1-3I) and acetyl Co-A oxidase 3 (Acox3). In summary, all endpoints showed that ATV, EE2 and their combination disrupted lipid metabolism in brown trout after in vivo exposures, uncovering specific effects of the MIX.

Acknowledgements. ICBAS-U.Porto; Project ATLANTIDA (NORTE-01-0145-FEDER-000040), by NORTE 2020, under PORTUGAL 2020 (through ERDF); FCT (UIDB/04423/2020 and UIDP/04423/2020 to CIIMAR).

ORAL COMMUNICATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

DIETARY LIPID SENSING THROUGH FATTY ACID OXIDATION AND CHYLOMICRON FORMATION IN GASTROINTESTINAL TRACT OF RAINBOW TROUT

Calo J*, Blanco AM, Comesaña S, Fernández-Maestú C, Alborja-Valado M, Chivite M, Conde-Sieira M, Soengas JL.

Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo. *Email: jessica.calo.rodriguez@uvigo.gal

In mammals, the inhibition of fatty acid oxidation (FAO) stimulates eating, highlighting the importance of energostatic mechanisms in food intake control. In fish, lipids are important dietary nutrients responsible for many physiological processes, and their gastrointestinal detection can modulate feed intake via the gut-brain axis. In the current study, using rainbow trout as a teleost model, we aimed to characterize, for the first time in fish, the role of FAO and chylomicron formation as peripheral lipid sensors, different from gut receptors, that could modulate feed intake. In the first experiment, we fed fish with either a normal-fat (24%; 2 tanks) or a high-fat (32%; 2 tanks) diet for 10 days. Then, to fish of both dietary treatments, we intraperitoneally (IP) administered 500 µL/100 g bw of water alone (control) or containing 2.5 µg/g of etomoxir to determine the effect of Cpt1 inhibition on feed intake at 6, 24, 48 and 72h after treatment. Feed intake results showed a significant decrease at 24h upon etomoxir treatment regardless the dietary condition, an effect that disappeared at 48 and 72h. In the second experiment, we sampled fish to evaluate changes in the levels of plasma metabolites (glucose, free fatty acid and triglycerides), Cpt1 enzyme activity and its mRNA relative abundance, as well as changes in the mRNA relative abundance of genes related to fatty acid transport, chylomicron formation and lipid metabolism in the proximal and posterior intestine and liver. Moreover, we analysed changes in feeding-regulating hypothalamic neuropeptides. Results are discussed in the context of peripheral metabolic regulation of feed intake in fish.

Acknowledgements. This work was supported by a research grant from the Spanish Agencia Estatal de Investigación and European Fund of Regional Development (PID2019-103969RB-C31) and Xunta de Galicia (Consolidación e estructuración de unidades de investigación competitivas do SUG, ED431B 2019/37) to JLS.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

OBESITY IMPAIRS COGNITIVE FUNCTION WITH NO EFFECT ON ANXIETY-LIKE BEHAVIOUR IN ZEBRAFISH

Godino-Gimeno A[1], Chivite M[2], Míguez JM[2], Winkberg S[3], Cerdá-Reverter JM[1]*

[1]Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, 12595 Castellon, SPAIN. [2]Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, 36310 Vigo, Spain. [3]Behavioural Neuroendocrinology, Department of Neuroscience, Uppsala University, 751 24 Uppsala, Sweden.

*Email: jm.cerda.reverter@iats.csic.es.

Obesity is a major challenge to health care systems in western societies and also a major contributor to metabolic syndrome. These metabolic disorder has profound negative effects on brain structure, impairing cognitive function and emotional states, exacerbating the development of learning/memory dysfunction and promoting anxiety-related responses. In the last decade, zebrafish has become an important model organism for studying behavioural and neurological disorders as well as metabolic diseases. This makes zebrafish an interesting model to study the effects of energy impairments on behavioural aspects. Here, we study the effects of overfeeding-induced obesity (OIO) on anxiety-like behavior and cognitive processes in zebrafish. The experimental animals were fed 4 times more food than control fish for 8 weeks and then anxiety-like behaviour was tested using novel tank test (NTT, n=24). Subsequently learning capacity, short- (STM) and long-term memory (LTM) were tested using aversive learning test in Zantik's units (n=24). Finally, fish were euthanised and biometrically sampled. Whole-body samples (n=8) were taken for total lipids content. In addition, brains (n=8) were dissected for monoamine HPLC determination. Results show that OIO has no effect on anxiety-like behaviour and LTM acquisition but impairs STM regardless of the fish gender, revealing the obesity effects on cognitive processes in zebrafish. Results suggest that these effects are dependent on food protocols and/or diet composition. Obese fish exhibited no deficiency of monoaminergic transmission revealed by the quantification of total brain levels of dopamine and serotonin and their metabolites. A reliable protocol is provided to assess the effect of metabolic diseases on cognitive and behavioural function thus supporting zebrafish as a model in behavioural and cognitive neuroscience.

Acknowledgements. This study was financed by funds from MCIN/AEI/ 10.13039/501100011033 (PID2022-136288OB-C33) and, as appropriate, by "ERDF A way of making Europe", by the "European Union" or by the "European Union Next Generation EU/PRTR".

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

ASPROSIN A POTENTIAL ROLE IN THE REGULATION OF ENERGY BALANCE IN FISH

Leal E*, Angotzi R, Cerdá-Reverter JM.

Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, 12595 Castellon, SPAIN. *Email: esther@iats.csic.es

Asprosin (Asp) is encoded in the carboxyl-terminal region of fibrillin 1 (FBN1), an essential protein for the formation of elastic fibres in connective tissue. Missense mutations are responsible for Marfan syndrome, which promotes poor appetite, thinness, bone growth and hypotonia. Asp is processed from the FBN1 precursor and is secreted from adipose tissue into the bloodstream at minimal levels after ingestion. It crosses the blood-brain barrier to activate orexigenic AGRP hypothalamic neurons in a cAMP-dependent manner and inhibits anorexigenic POMC hypothalamic neurons through a GABA-dependent pathway, stimulating ingestion and reducing metabolic output. Mutant mice for FBN1 show a similar phenotype and administration of Asp rescues the wild-type phenotype. Obese individuals have elevated levels of Asp, and neutralisation with monoclonal antibodies reduces intake and obesity. An Asp receptor with orexigenic activity (Ptprd) has recently been described. These results suggest that Asp may be an energy transducer from the periphery to the central energy systems, in particular to the melanocortin system. Our preliminary data show that Asp is evolutionarily conserved and suggest that it is expressed in adipose tissue from very early developmental stages. The evolutionary conservation and expression patterns of Asp suggest that Asp may be an important peptide in the regulation of food intake in fish, transducing energy information from adipose tissue and using the melanocortin system as a central effector. Our recent studies in zebrafish have shown that the expression levels of Ptprd and asprosin are modulated depending on the physiological state of the fish (fasting or overfeeding).

Acknowledgements. Funded by European Social Fund (FSE) of Generalitat Valenciana, project APOSTD and bySpanish Science and Innovation Ministry, project PID2022-136288OB-C33.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CORTISOL MODULATION OF METABOLIC CONTROL OF FEED INTAKE IN RAINBOW TROUT HYPOTHALAMUS

Comesaña S[1]*, Calo J[1], Conde-Sieira M[1], Maestú CF[1], Alborja-Valado M[1], Soengas JL[1], Vijayan MM[2], Blanco AM[1].

[1]*Centro de Investigación Mariña (CIM), Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, 36310 Vigo, Spain. [2]Department of Biological Sciences, University of Calgary, Calgary, AB, Canada. *Email: saracomesana@uvigo.es

Feed intake regulation is a complex physiological process involving many signals that are integrated in the hypothalamus. Among these regulating signals, the levels of circulating nutrients, known as metabolic information, are critical to maintain energy homeostasis. However, under stress conditions, this homeostasis is disturbed resulting in decreased feed intake. Previous studies in fish, demonstrated that stress affects metabolic regulation of feed intake by glucose, but there is no available information for other nutrients. Thus, we aimed to study how stress, through increasing cortisol levels, affects the metabolism in hypothalamus and its implication in the regulation of food intake. For this, rainbow trout (*Oncorhynchus mykiss*) were treated with intraperitoneal coconut oil implants as control (5 µL/g body weight) or containing cortisol (50 µg/g body weight). Then, feed intake levels were evaluated for 3 days in three replicates. Experiment was repeated but this time on the third day, 12 fish per group were not fed in order to obtain different brain regions: hypothalamus, forebrain and distal brain. We analyzed mRNA levels of specific transcripts related to feed intake control, metabolism and nutrient sensing. In addition, we quantified levels of different metabolites through metabolomics analysis. The obtained results showed that cortisol affected different central metabolic pathways, highlighting the relevance of metabolic regulation of feed intake regulation.

Acknowledgements. This work was supported by a research grant from the Spanish Agencia Estatal de Investigación and European Fund of Regional Development (PID2019-103969RB-C31) and Xunta de Galicia (Consolidación e estructuración de unidades de investigación competitivas do SUG, ED431B 2019/37) to JLS.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

IMPACT OF DIETS FORMULATED WITH INSECT MEAL ON THE FEEDING BEHAVIOUR OF ATLANTIC SALMON FRY

Chivite M[1,2]*, Clokie B[2], MacKenzie S[2], Santos S[2], Rey Planellas S[2].

[1]*Centro de Investigación Mariña (CIM), Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, Pontevedra, Vigo, 36310, Spain. [2]Institute of Aquaculture (IoA), University of Stirling, Stirlingshire, FK9 4LA, United Kingdom. *Email: mchivite@uvigo.gal

The black soldier fly larva (BSFL, *Hermetia illucens*) meal is attracting interest due to its efficiency in converting devalued substrates into high-quality protein through circular economies. In addition, BSFL-meal contains appropriate nutritional profiles along with functional compounds that may potentially improve the welfare of fish. However, there is a lack of knowledge about its impact on fish behaviour, which is fundamental to achieve high welfare standards and full understanding of their effects on salmon performance. This study evaluated the feeding behaviour of Atlantic Salmon fry (*Salmo salar*) conditioned from their first feeding stages to be fed on 4 different diets, isocaloric and isoproteic, but formulated with different inclusion of defatted BSFL-meal (0, 8, 16 and 24%) for 21 days. Each day, four 30min video clips were recorded (00, 06, 12 and 24h) and analyzed with EthoVision® XT (Noldus, Wageningen, Netherlands) and Multiviewer software (Computer System Department, University of Murcia, Spain). An ethogram was built to describe the suit of different behaviours observed and a group scan sampling was used to analyse the videos. The results were adjusted to a sinusoidal curve to detect potential patterns of circadian rhythms. Significant differences were found in preference zones, average speed or distance moved, indicating that fish fed with BSFL-meal were more active and spend less time in the feeding zone (surface layer). These results suggest that BSFL-meal promotes a pattern compatible with darting-like behaviour, a more natural feeding strategy, which in turn shows a prominent circadian component. Behavioural measures should be considered when designing new feeding protocols and further research is needed to determine the implications for fish positive welfare.

Acknowledgements. Study funded by Innovate UK (RUK IFS 47278) to Professor Simon MacKenzie at the IoA, University of Stirling. Chivite, M. was the holder of a postdoctoral grant from the Xunta de Galicia (ED481B-2022-082).

POSTER PRESENTATIONS

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

MODULATION OF STRESS RESPONSES AND METABOLISM THROUGH THE DIET: A NATURAL ADDITIVE TO IMPROVE FISH RESILIENCE

Cartan S, Pérez-Peña M, Martos-Sitcha JA, Mancera JM, Jerez-Cepa I*.

Department of Biology, Faculty of Marine and Environmental Sciences, Institute of Marine Research (INMAR), University of Cádiz, Puerto Real, Spain. * Email: ismael.jerez@uca.es

Handling, transport or high stocking density are common practices in aquaculture that activate the stress response and induce cortisol release, the main stress hormone in teleost fish. Stress attenuation in farmed fish is essential to ensure productivity since it improves both general immune status and growth rates. Thus, the development of the aquaculture industry must be linked to animal welfare improvement. Dietary supplements are a potential solution to reduce the negative effects of stress. This study assesses the effectiveness of a natural additive with sedative properties on seabass juveniles (*Dicentrarchus labrax*) submitted to high stocking density stress conditions and a transport situation. Seabass juveniles were placed into 500 L-tanks and distributed in four different experimental groups in triplicate, defined as the combination of feeding (control or supplemented diet) and the initial stocking density (low density: 4 kg/m^3 or high density: 18 kg/m^3). Fish were maintained for 70 days under these conditions and afterward were sampled. Subsequently seabass juveniles were placed into 15 L-tanks for a 3 h-transport simulation. At the end of the transport, half of the animals from each group were euthanized and sampled. The remaining fish were transferred into clean water tanks to determine their status after 24 h, of recovery. Changes on plasmatic primary (cortisol) and secondary (metabolites) stress responses, as well as changes on intermediary metabolism pathways in liver were determined in both stressing experiments. Cortisol levels confirmed the activation of stress endocrine axis, which was modulated by the addition of the natural additive. These results, in concordance with metabolic responses, shows the potential of these natural solutions to improve fish resilience in aquaculture.

Acknowledgements. Proyecto PISCIBIEN. Funded by European Union-Next Generation. PRTR, Ministerio de Agricultura Pesca y Alimentación (MAPA, España).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

The peptide GHRP-6, a ghrelin analog, administered through food modulates the endocrine and metabolic response of *Sparus aurata* to IFA treatment

Rodríguez-Viera L[1], Martí I[1], Martínez R[2], Perera E[3], Mancera JM[1], Martos-Sitcha JA[1]*.

[1]*Department of Biology, Faculty of Marine and Environmental Sciences, Instituto de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), University of Cádiz, Spain. [2]Aquatic Biotechnology Department, Biotechnology Animal Division, CIGB, Havana, Cuba. [3]Instituto de Ciencias Marinas de Andalucía, Consejo Superior de Investigaciones Científicas (ICMAN-CSIC), Puerto Real, Cádiz, Spain.
*Email: juanantonio.sitcha@uca.es

The aquaculture sector has experienced an important growth with the subsequent increase of feeding and nutritional issues for sustaining this activity, mainly related to the use of high quality, safe and environmentally friendly feed ingredients. The use of additives in aquafeeds has proven to be a suitable option to improve different productive indicators in farmed fish. In this study, the effect of inclusion of the GHRP-6 peptide (500 µg/kg of feed), a ghrelin analog, in a commercial diet (CTRL) of gilthead sea bream (*Sparus aurata*) was studied in a medium-term feeding trial. After 97 days, fish were intraperitoneally injected with 100 µL of saline solution (SS) or Incomplete Freund's adjuvants (IFA)/100 g fish, and then sampled for blood and tissue at 72 h post-injection. Previously, it was found that inclusion of GHRP-6 in aquafeeds significantly enhanced plasma Gh levels (in agreement with the Gh secretagogue effects of ghrelin) as well asgrowth performance and feed efficiency. CTRL fish showed a significant increase in lactate and glucose values when challenged with IFA (CTRL/IFA), whereas plasma triglycerides decreased. Furthermore, fish fed with GHRP-6 diet did not show differences between both injected subgroups (GHRP-6/SS and GHRP-6/IFA). Moreover, animals from GHRP-6/IFA group decreased circulating cortisol values respect to CTRL/SS group, suggesting a possible protective effect by GHRP-6 peptide. Interestingly, cortisol and cholesterol values were significantly higher in fish from GHRP-6/SS group. Taken together, these preliminary results indicate that this peptide is a viable dietary supplement to increase production efficiency of *S. aurata*, and also suggest a protective effect on the fish's immune system in this specie.

Acknowledgements. We acknowledge of Servicios Centrales de Investigación en Cultivos Marinos (UCA) and Experimental Feeds Service (UAL) for their support on animal care and aquafeed elaboration. This research was supported under the Fellowship “Ayudas María Zambrano para la atracción del talento internacional” awarded by the University of Cadiz (UCA/R155REC/2021).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CORTISOL DYNAMIC IN WATER AND DIFFERENT BIOLOGICAL MATRICES IN GILTHEAD SEA BREAM SPECIMENS UNDER DIFFERENT STOCKING DENSITIES AND FEEDING REGIMES

Sánchez-Ruiz D[1], Salas A[1], de las Heras V[2], Molina-Roque L[1], Simó-Mirabet, P[1], Mancera JM[1], Martos-Sitcha JA[1]*.

[1]*Department of Biology, Faculty of Marine and Environmental Sciences, Instituto de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), University of Cádiz, Spain. [2]Servicios Centrales de Investigación en Cultivos Marinos (SCI-CM), University of Cádiz, Spain.

*Email: david.sanchezruiz@uca.es

In the world population there is a generalized growing awareness about animal welfare in culture activities that is promoting research and innovation in issues related to stress and health care in aquaculture. Stress is induced by poor conditions in the farming (low water quality, crowding, inappropriate handling...) conditioning a decrease in appetite, low feed efficiency and feed conversion ratios. Traditionally, cortisol in plasma, the end product of the hypothalamus-pituitary-interrenal (HPI) axis, has been measured as a well biomarker of stress in fish, but attempts are being made to measure cortisol in other matrices less invasive than blood extraction, such as cutaneous mucosa, skin scales and culture water. The objective of this study was to evaluate the dynamic of this hormone in different matrices in a medium-term trial performed with gilthead sea bream (*Sparus aurata*) juveniles maintaining at different stocking densities and discriminating the metabolic orchestration derived from the reduction of food intake. For this, fish of ~31.3 g were submitted to 3 different treatments, in triplicate, for 90 days: i) low stock density fed *ad-libitum* (LSD), ii) high stock density fed *ad-libitum* (HSD), and iii) low stock density pair feed with the HSD (LSD-PF). Our results demonstrate that growth performance was reduced in HSD due to a combination of stress by crowding and lower ingestion rates, although growth in LSD-PF fish was similar to that observed in LSD thanks to an improvement in feed efficiency. Finally, HSD induced an increase in plasma cortisol respect to the other two treatments, being also consistent in the other matrices, with mucus showing the highest correlation with plasma cortisol.

Acknowledgements. This study was supported by the Project IRSAF (PID2020-117557RB-C22, Ministerio de Ciencia e Innovación, Spain.

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

EMERGING STRESSORS IN DEEP-SEA ENVIRONMENTS: CASE STUDIES USING HYPERBARIC HYPERBARIC CHAMBERS FOR HAZARD AND RISK ASSESSMENT OF DEEP-SEA MINING

Pinheiro M[1,2], Marassi R[1], Oliveira A[1,2], Barros S[1], Alves N[1,2], Morais H[1,2], Ribeiro M[1], Raimundo J[1,3], Caetano M[1,3], Neuparth T[1], Santos MM[1,2]*.

[1]CIIMAR/CIMAR-LA – Interdisciplinary Centre for Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos s/n, 4450-208 Matosinhos, Portugal. [2]FCUP – Biology Department, Faculty of Sciences of the University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal. [3]IPMA – Portuguese Institute for Sea and Atmosphere, Avenida Alfredo Magalhães Ramalho 6, 1495-165 Algés, Portugal. *Email: miguel.santos@fc.up.pt.

The deep-sea, despite its remoteness, is subjected to anthropogenic stressors, among which, deep-sea mining. Deep-sea mining is being looked at with renewed interest due to increased need for minerals and rare earth elements that are depleting land-based deposits because of technological advances, mainly green energy. Deep-sea mining will release sediment plumes, considered one of the most extensive and immediate effects as they can travel for hundreds of kilometers across the water column. To study the potential effects of suspended sediments, we used two model species, *Mytilus galloprovincialis* mussel and *Spisula solida* clam, exposed for 96h in a hyperbaric chamber, to different sizes of sediments (63-125;125-250;250-500μm) in a mixture with different concentrations (1, 2 and 4g/L) at increasing pressures (up to 50Bar). We analyzed the filtration rate (FR) and oxidative stress biomarkers in mussels and clams. The FR decreased in all conditions and significant changes were observed in all tested biomarkers. We also studied the effects of the different size classes of sediments separately by exposing juvenile mussels to the different sizes of sediments at 1g/L and 4Bar. Molecular, biochemical and functional endpoints were analyzed. FR decreased significantly for all sizes with a more pronounced effect in smaller particles. Significant changes were found in all tested biomarkers and gene expression was altered in genes related to oxidative stress. Sediments affected organisms at all levels, with a more pronounced effect for smaller particles. As such, particular attention should be given to their release as a by-product of mining. These findings help to fill knowledge gaps in the effects of suspended particles, contribute to hazard and risk assessment of deep-sea mining and to establish frameworks to mitigate effects of this stressor.

Acknowledgements. Marlene Pinheiro acknowledges FCT for the PhD Grant SFRH/BD/147834/2019. This work was supported by the projects DeepRisk [PTDC/CTA-AMB/7948/2020] and Atlantida [NORTE-01-0145-FEDER-000040].

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

CHARACTERIZATION OF THE MAIN FAT DEPOTS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

Rosell-Moll E, Balbuena-Pecino S, Rodríguez I, Monblanch M, Kim-My NT, Garcia de la serrana D, Gutiérrez J, Capilla E, Navarro I*.

Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona 08028, Spain. *Email: mnavarro@ub.edu

Increased visceral adiposity is undesired in aquaculture fish as it can affect not only fat metabolism, but also product yield. However, other adipose tissue depots less characterized such as the intramuscular, can also contribute importantly to the quality of the final product. In Spain, the primary freshwater fish species produced is the rainbow trout (*Oncorhynchus mykiss*), and the visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and intramuscular adipose tissue (IMAT) are their main fat depots. This study highlights the morphological and metabolic differences among these adipose tissue depots through histological and transcription approaches. By histological examination, adipocytes from VAT and SAT were found to be bigger and less abundant compared to IMAT adipocytes in agreement with the primary role of VAT and SAT as fat storage sites. Conversely, IMAT cells were smaller and more abundant near specific areas in the myoseptum, accordingly to the function of this depot as a source of energy for demanding muscular activities. Histological data also indicated that young fish (300 g) primarily experiences hyperplastic growth in their adipose tissues, while mature fish (2 Kg) displays predominantly hypertrophic development in agreement to the observed tendency to accumulate fat with age. Moreover, adipokines, adipogenesis and lipid metabolism-related genes expression was analyzed for VAT and SAT, and results indicated differences exist between these two fat depots. Specifically, in young rainbow trout, higher expression of genes involved in β-oxidation and fatty acid transport (i.e., *pparα* and *fabp11α*, respectively) was found in VAT adipocytes. Altogether, data suggest that VAT exhibits higher metabolic activity than SAT, especially at young ages. Additionally, with the recent achievement of mature adipocytes isolation from SAT and IMAT, a more comprehensive understanding of the differences and similarities among these adipose tissue depots, including VAT, will be accomplished.

Acknowledgements. Supported by MICINN (PID2020-116172RB-I00) and GenCat (2021FISDU-00314).

XIV Congress of the Iberian Association of Comparative Endocrinology

Bilbao, 11-13 September 2023

MODULATION OF STRESS RESPONSES AND METABOLISM THROUGH THE DIET: A NATURAL ADDITIVE TO IMPROVE FISH RESILIENCE

Cartan S, Pérez-Peña M, Martos-Sitcha JA, Mancera JM, Jerez-Cepa I*.

Department of Biology, Faculty of Marine and Environmental Sciences, Institute of Marine Research (INMAR), University of Cádiz, Puerto Real, Spain. *Email: ismael.jerez@uca.es

Handling, transport or high stocking density are common practices in aquaculture that activate the stress response and induce cortisol release, the main stress hormone in teleost fish. Stress attenuation in farmed fish is essential to ensure productivity since it improves both general immune status and growth rates. Thus, the development of the aquaculture industry must be linked to animal welfare improvement. Dietary supplements are a potential solution to reduce the negative effects of stress. This study assesses the effectiveness of a natural additive with sedative properties on seabass juveniles (*Dicentrarchus labrax*) submitted to high stocking density stress conditions and a transport situation. Seabass juveniles were placed into 500 L-tanks and distributed in four different experimental groups in triplicate, defined as the combination of feeding (control or supplemented diet) and the initial stocking density (low density: 4 kg/m^3 or high density: 18 kg/m^3). Fish were maintained for 70 days under these conditions and afterward were sampled. Subsequently seabass juveniles were placed into 15 L-tanks for a 3 h-transport simulation. At the end of the transport, half of the animals from each group were euthanized and sampled. The remaining fish were transferred into clean water tanks to determine their status after 24 h, of recovery. Changes on plasmatic primary (cortisol) and secondary (metabolites) stress responses, as well as changes on intermediary metabolism pathways in liver were determined in both stressing experiments. Cortisol levels confirmed the activation of stress endocrine axis, which was modulated by the addition of the natural additive. These results, in concordance with metabolic responses, shows the potential of these natural solutions to improve fish resilience in aquaculture.

Acknowledgements. Proyecto PISCIBIEN. Funded by European Union-Next Generation. PRTR, Ministerio de Agricultura Pesca y Alimentación (MAPA, España).