8TH GENOMICS IN AQUACULTURE • SPLIT 2026

THANK YOU FOR JOINING US

*Advancing the science of aquaculture genomics*

WEB jadran.izor.hr/gia2026

EMAIL gia2026@izor.hr

**Institute of Oceanography and Fisheries**
Institut za oceanografiju i ribarstvo • Split, Croatia

SPLIT • CROATIA • 2026

Genomics *in Aquaculture* 8TH EDITION

8TH EDITION

# Genomics *in Aquaculture*

DATES
**4-6 May 2026**
Study visit • 7 May

LOCATION
**Split, Croatia**
Spinit Technology Park

EDITION
**GIA 2026**
jadran.izor.hr/gia2026

**BOOK OF ABSTRACTS** Hosted by the **Institute of Oceanography and Fisheries**, Split

**Institute of Oceanography and Fisheries**
Institut za oceanografiju i ribarstvo • Split, Croatia

SPLIT • CROATIA • 2026

## WELCOME

*We are delighted to welcome you to Split for the 8th edition of Genomics in Aquaculture.*

*Once again, you have placed your trust in GIA, creating a vibrant gathering where we can share the latest advances in genomics research among colleagues and friends. We sincerely thank you all—your continued support is what keeps GIA growing and thriving.*

*We have worked hard to prepare an engaging and inspiring programme, and we hope you will enjoy not only the science, but also the social events and the unique atmosphere of Split.*

*We look forward to meeting you all in Split.*

*GIA 2026 Organizing & Scientific Committees*

**The Organising Committee**

Jorge Fernandes, Institute of Marine Sciences, Spain

Elena Sarropoulou, Hellenic Centre for Marine Research, Greece

Ivana Bušelić Garber, Institute of Oceanography and Fisheries, Croatia

Diego Robledo, The University of Edinburgh, UK

Ivana Lepen Pleić, Institute of Oceanography and Fisheries, Croatia

Igor Talijančić, Institute of Oceanography and Fisheries, Croatia

Arun Mattanavile Shankregowda, Swansea University, UK

**The Scientific Committee**

Jorge Fernandes, Institute of Marine Sciences, Spain

Elena Sarropoulou, Hellenic Centre for Marine Research, Greece

Ivana Bušelić Garber, Institute of Oceanography and Fisheries, Croatia

Diego Robledo, The University of Edinburgh, UK

Kiron Viswanath, Nord University, Norway

Zhanjiang (John) Liu, Tennessee Technological University, USA

Tanja Šegvić Bubić, Institute of Oceanography and Fisheries, Croatia

Željka Trumbić, University of Split, Croatia

Sara Faggion, University of Padova, Italy

**Book of Abstracts Editor**

Ivana Bušelić Garber, Institute of Oceanography and Fisheries, Croatia

# Genomics
# *in Aquaculture*

**8TH EDITION** CONFERENCE

# INDEX

# INVITED SPEAKERS

## Dr. Gen Hua Yue

*Temasek Life Sciences Laboratory, Singapore*

Dr. Gen Hua Yue is Senior Principal Investigator and Program Director at Temasek Life Sciences Laboratory, Singapore, leading the Molecular Population Genetics and Breeding Group. He earned his B.Sc. and M.Sc. from Nanjing Agricultural University and Ph.D. in Agricultural Sciences at the University of Hohenheim, Germany.

Since 1998, he has advanced molecular breeding, aquaculture genetics, and plant genomics across various species, including Asian seabass, tilapia, oil palm, and sugarcane. Dr. Yue has secured over SGD 38 million in funding, published more than 220 peer-reviewed papers, serves on 10+ editorial boards, reviewed international research programs, held adjunct professorships at NUS and NTU, and delivered over 100 invited talks globally.

**Keynote — Day 1**

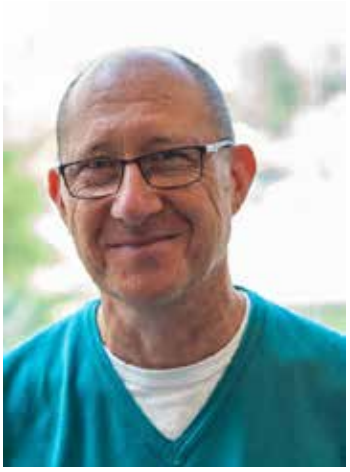
## Dr. Kaja Helvik Skjærven

*Institute of Marine Research, Norway*

Dr. Kaja H. Skjærven is a senior researcher at the Institute of Marine Research in Norway, with a special interest in fish nutrition, embryonic development, and epigenetic regulation. With a degree in environmental physiology, she investigates how factors like diet, temperature, and contaminants influence DNA methylation and gene regulatory mechanisms across generations in salmon, cod, and zebrafish.

Her projects especially explore how environmental factors influence tissue nutritional status during maturation and how parental environments shape offspring phenotypes. Recent publications highlight intergenerational effects of spawning season, ocean warming, and nutrient availability, providing insight into how fish adapt to climate-driven environmental changes and evolving aquaculture practices.

**Keynote — Day 2**



## Dr. Yann Guiguen

*Fish Physiology and Genomics Laboratory,*

*INRAE Brittany-Normandy, France*

Dr. Yann Guiguen is a Research Director at the French National Research Institute for Agriculture, Food, and Environment (INRAE), based in Rennes, Brittany, France. He is a "physio-genomicist" who uses molecular and genomics approaches to investigate the evolution of sex determination and sex differentiation mechanisms in fish.

Yann combines genomics and large-scale whole genome sequencing projects with functional and expression-based approaches to explore the evolution of sex chromosomes and master sex determination genes. He is widely recognised in the fish community for his contributions to the endocrinology of gonadal sex differentiation, the evolution of sex determination systems, and the discovery of an unusual sex determining gene in salmonid species.

**Keynote — Day 3**

# ORAL PRESENTATIONS

## DAY 1 — Monday, 4 May 2026

### Session 1 — Immunity, stress & welfare I

**Chairs:** Lior David, Carlo Lazado

# LONG-READ GRAPH GENOME OF SEA BREAM HIGHLIGHTS STRUCTURAL VARIANT ENRICHMENT IN GENES DRIVING ADAPTIVE PLASTICITY

Hernández-Juarez, A. [1,2], Naya-Català, F. [1], Lien, S. [3], Kent, M. [3], Falcó, A. [1] and Pérez-Sánchez, J. [1]

*[1]Instituto de Acuicultura Torre de la Sal, Castellón, Spain; [2]Universitat Politècnica de València, Valencia, Spain; [3]Norwegian University of Life Sciences, Ås, Norway.*

e-mail: aldo.hernandez@csic.es

While short-read sequencing often misses complex structural variants (SVs), long-read sequencing (LRS) and graph-based pangenomics offer a superior resolution for genomic diversity, yet their application in marine fish populations remains very limited. In this study, we present an LRS-based graph genome for a population of 13 gilthead sea bream (*Sparus aurata*) individuals selected for their thermal resilience and fast growth. Using the PromethION 24 platform (42× coverage, 13.3 kb average read length), we generated high-quality individual assemblies (N50: 30–37 Mb; >98% gene completeness) and constructed a graph genome with Minigraph-Cactus, detecting a total of ~229k SVs and therefore exceeding structural diversity values[1] reported for other farmed species. In previous studies, we identified 3,114 candidate thermal-responsive genes (TRGs) and 171 biological age marker genes (BAGs), by linking epigenetic and transcriptomic landmarks in muscle samples[2,3]. While ~35% of the total gene set contains at least one SV, this percentage increases up to ~44% in TRGs and ~61% in BAGs (Fisher test, $p < 0.001$). This SV-enrichment suggests that these genes, potentially involved in the population's adaptive plasticity, act as hotspots for structural variations. Focusing on a subset of 33 genes (23 TRGs and 10 BAGs), fully validated as markers of adaptive thermal responses and acceleration/deceleration of biological age, we found out that, despite their SV-enrichment, they are mostly located in chromosomal regions with low overall SV density (Figure 1). These findings highlight that LRS-based graph genomics are emerging as powerful tools to uncover the structural mechanisms underlying thermal resilience and adaptive plasticity in a context of global warming and intensified aquaculture production.

**Keywords**: *Sparus aurata*, Long-read sequencing, Genome graph, Structural variant

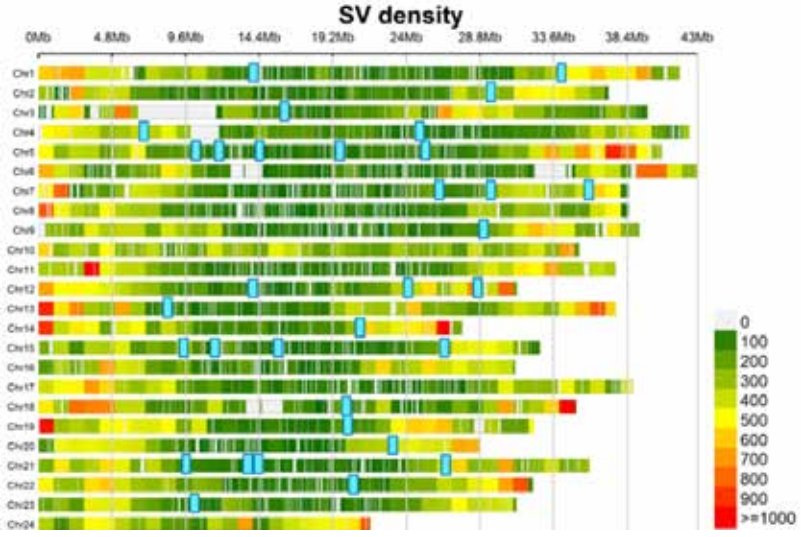



***Figure 1.*** *SV density per 1 Mb intervals along different chromosomes. In blue are highlighted the 33 adaptive plasticity genes*

## *Reference*

1. Loegler et al. (2026). Cell Genom 6, 10;
2. Belenguer et al. (2024). IJMS 25, 9836;
3. Naya-Català et al. (2025). EPIMAR2025.

# RNA SEQUENCING REVEALS MARKERS OF IMMUNITY IN ATLANTIC SALMON (*SALMO SALAR*) VACCINATED AGAINST *TENACIBACULUM FINNMARKENSE*

Florea, A. [1], Mechlaoui, M. [2], Haganes, T. S.[3], Bean, T. P. [1] and Seternes, T.[2,]

*[1]The Roslin Institute, Edinburgh, Scotland, [2]The Arctic University of Norway (UiT), Tromsø, Norway, [3]Vaxxinova Norge, Bergen, Norway.*

e-mail: alexandra.florea@ed.ac.uk

Fish viral and bacterial diseases cause substantial losses for the Norwegian aquaculture sector. While vaccination and selective breeding provide protection against many of these threats, there are still a number for which no direct preventative measures are available including one causal agent of ulcerative skin disease, *Tenacibaculum finnmarkense*. The aim of study is to evaluate the effectiveness and mode of action of monovalent inactivated vaccines *against T. finnmarkense* in Atlantic salmon (*Salmo salar*). For this, three different vaccine regimes comprising both water-based immersion and oil- based IP vaccines were tested and immune system metrics assessed at various points in the vaccination process.

Initial results demonstrated a specific IgM response against *T. finnmarkense* in all IP vaccinated fish and significant differences in mortality were observed between unvaccinated control fish and vaccinated fish. Bulk RNA-sequencing on samples from different timepoints of our best vaccination group, using 100 head kidney and thymus samples. This has allowed us to characterise the immune and transcriptional response of Atlantic salmon against this *T. finnmarkense* vaccine, the acute stress response of a placebo vaccination, and to identify biomarkers associated with this response.

**Keywords**: RNA-Seq, Vaccination, Teleost, Salmon, Bacteria

# DISEASE RESISTANCE MECHANISMS AND INFECTIVITY ARE DIFFERENT BETWEEN COMMON CARP AND RELATED CYPRINIDS

David, L.[1], Dorfman, B.[1], Lamichhane S.[2], Marcos-Hadad, E.[1] and Gorgoglione, B. [2]

*[1]Department of Animal Sciences, RH Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel; [2]Dept. Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA*

e-mail: lior.david@mail.huji.ac.il

Common carp (*Cyprinus carpio*) is among the most widely produced aquaculture species. Outbreaks of a disease caused by cyprinid herpes virus type 3 (CyHV-3) have been significantly damaging its production worldwide. Our group has been breeding for CyHV-3 disease resistant strains and showed that our common carp fish are disease resistant because they are infected by the virus, but do not die due to their improved immunity. Common carp and other related cyprinids are often found together in natural water bodies and certainly are grown together in aquaculture. We wanted to study interactions between common carp and related cyprinids with respect to disease resistance and transmission. Firstly, we injected common carp fish with virus and tested if they can infect by cohabitation other cyprinids, namely, grass carp, black carp, silver carp and goldfish. Almost no mortalities were observed in other cyprinids, however, a small proportion of these fish were PCR positive for the virus, suggesting that other cyprinids have a CyHV-3 infection resistance mechanism. Therefore, to overcome the infection barrier, we continued to a second experiment, in which other cyprinids were CyHV-3 injected. Upon forced infection, significant viral loads in spleen were PCR detected in all cyprinids. However, compared to common carp, other cyprinids cleared the virus faster and suffered no mortalities, suggesting also an improved disease resistance mechanism. Lastly, cohabitation of common carp with CyHV-3 injected fish, indicated that other cyprinids are much less infective than common carp. Taken together, these other cyprinids presented both infection and disease resistance mechanisms, suggesting that they are inefficient secondary hosts, which slightly increase disease outbreak risks in common carp aquaculture, but less so in natural water bodies.

**Keywords**: Carps, Cyprinid herpes virus type 3, Koi herpes virus, Disease resistance, Infectivity, Epidemiology

# INSIGHTS INTO THE GENETIC ARCHITECTURE OF RESISTANCE TO VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN RAINBOW TROUT FROM GWAS AND FUNCTIONAL VALIDATION

Thomas, V. [1], Collet, B. [2], Boudinot P. [2], Phocas F. [1], and Lallias D. [1]

[1] *Université Paris-Saclay, INRAE, GABI, 78350 Jouy-en-Josas, France,* [2] *Université Paris-Saclay, INRAE, UVSQ, VIM, 78350 Jouy-en-Josas, France*

e-mail: valentin.thomas@inrae.fr

Although aquaculture is one growing food production sectors worldwide, its sustainability is threatened by infectious diseases. Viral Hemorrhagic Septicemia (VHS) is a particularly severe disease affecting farmed rainbow trout (*Oncorhynchus mykiss*). Outbreaks not only diminish the productivity and profitability of salmonid aquaculture but also pose mortality risks to wild fish populations. Indeed, VHSV infects a wide range of wild freshwater and marine fish species. To investigate the genetic architecture and molecular mechanisms underlying survival to VHSV, 2500 offspring from a factorial cross of 32 dams and 60 sires from the INRAE synthetic line were challenged with waterborne VHSV infection. Parentage assignment was conducted for the first 276 dead fish and 276 survivors. From parents with a sufficient number of assigned offspring (11-23 per parent), 14 individuals were selected—seven susceptible and seven resistant—for whole-genome sequencing. The seven susceptible parents exhibited progeny survival rates of 0% to 20%, while the resistant parents had survival rates of 83% to 100%. After variant calling, a total of 13M quality-filtered SNP with at least three minor alleles across all individuals were retained. Subsequently, a genome-wide association study was performed using Fisher's exact test at each SNP position, incorporating corrections for multiple testing and the correlations among closely located SNP. This analysis identified four new suggestive SNP associated with survival to VHSV challenge in rainbow trout, located on chromosomes 6, 8, 17, and 32. Notably, one of these SNP (located on chr17) was found within a gene potentially involved in viral resistance, a paralog of *lrp1* (*low-density lipoprotein receptor-related protein 1*). The function of this gene has been further explored through *in-vitro* experiments. Three distinct *lrp1*$^{-/-}$ CHSE-EC cell lines were obtained by CRISPR-Cas9 genome editing and evaluated for their survival to VHSV challenge. The results indicated that the chr17 SNP effect on whole-organism survival cannot be attributed to epithelial cell resistance, as the knockout had no significant effect on survival rates.

**Keywords**: Rainbow trout, VHSV, GWAS, Functional validation, cell lines

# MICRORNA-MEDIATED REGULATION OF HOST RESPONSE TO VIRAL NERVOUS NECROSIS VIRUS IN EUROPEAN SEABASS

Rodríguez-Vázquez, R. [1], Mukiibi, R. [2,3], Ferraresso, S. [4], Franch, R. [4], Peruzza, L. [4], Dalla Rovere, G. [4], Radojicic, J. [5], Babbucci, M. [4], Bertotto, D. [4], Toffan, A. [6], Pascoli, F. [6], Peñaloza, C. [2,7], Houston, R.D. [7], Tsigenopoulos, C. S. [5], Bargelloni, L. [4] and Robledo, D. [1, 2]

*[1]Dept. Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela (USC), Santiago de Compostela, Spain; [2]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK; [3]Dept. of Animal Health, Behaviour and Welfare, Harper Adamas University, Newport, Shropsire, UK; [4]Dept. Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy; [5]Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, Greece; [6]Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Fish Diseases, Legnaro, 35020, Italy; [7]Benchmark Genetics, Roslin Innovation Centre, Edinburgh, EH25 9RG, UK.*

e-mail: raquelrodriguez.vazquez@usc.es

MicroRNAs (miRNAs) are key post-transcriptional regulators of antiviral immunity, modulating gene expression by binding to the 3′ untranslated regions (UTRs) of immune-related transcripts. However, their contribution to viral nervous necrosis (VNN) resistance in European seabass (*Dicentrarchus labrax*) remains unexplored. Here, we characterize for the first time the brain miRNome of seabass from three VNN-resistance genotypes (susceptible, intermediate, resistant) across two genetically distinct populations. Differential expression analysis revealed cluster-specific patterns, with resistant fish consistently showing lower expression of specific differentially expressed miRNAs. Among them, miR-199-5p emerged as a strong candidate, displaying significantly lower expression in resistant fish. Functional characterization identified two miRNA recognition elements (MREs) for miR-199-5p within the *ifi27l2a* gene 3′ UTR, flanking a SNP (Chr3:10,082,380) previously associated with the major QTL for VNN survival. Moreover, a strong negative correlation (r = −0.840) between miR-199-5p and *ifi-27l2a* transcript levels supported a post-transcriptional repression mechanism. Together, our findings propose a regulatory model in which miR-199-5p modulates *ifi27l2a* expression, influencing phenotypic variation in VNN resistance. This study provides novel insights into antiviral immune regulation in seabass and highlights miR-199-5p as a promising biomarker for selective breeding in aquaculture.

**Keywords**: Small non-coding RNAs, post-transcriptional regulation, antiviral immunity, disease resistance, *Dicentrarchus labrax*

# CRISPR-CAS9 EDITING AS A TOOL TO INTERROGATE GENE FUNCTION: ATLANTIC SALMON TRIM25 PARALOGS ANTI-VIRAL FUNCTION IN INFECTIOUS SALMON ANAEMIA VIRUS

Stewart, R.[1], Perez, N.,[2], Clark, T.[1], Manousi, D.[1], Naseer, S.[3], Raudstein, M.[1], Fraslin, C.[1], Tim Regan[1], Martin, S.A.M.[3], Orosa-Puente, B.[1,2], and Robledo, D.[1,2 *]

[1] *The Roslin Institute, Edinburgh, Scotland,* [2] *University of Santiago de Compostela, Santiago de Compostela, Spain*
[3]*Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen*

e-mail: r.f.stewart@sms.ed.ac.uk

In model species, the E3 ubiquitin ligase TRIM25 is a major component of the host antiviral response, acting as both a sensor of viral RNA and a signal transducer, activating the RIG-I-like receptor pathway. In the global economic heavyweight Atlantic salmon (*Salmo salar*), *trim25* presents as two copies, and has been identified in multiple viral and viral mimic challenges as a virus-induced gene, putatively part of the type I interferon pathway. However, to date its role in the host response against viral pathogens remains uncharacterised. Viruses constantly threaten marine-raised Atlantic salmon, and novel strategies for prevention and treatment of viral outbreaks are fundamental for more efficient production. The purpose of this project is to characterise the function of the *Salmo salar trim25* to better understand the host response to viruses, potentially leading to improvements in the management of viral diseases in salmon farming. Using Atlantic salmon head kidney 1 (SHK-1) cells and Infectious Salmon Anaemia Virus (ISAV) infection as a model, we show ohnolog-specific, virus-inducibility and antiviral properties of *trim25*. While structural analyses showed that the two copies of *trim25* retain their functional domains, using CRISPR/Cas genome editing we have demonstrated that only the copy in Ssa02 has an impact on viral replication. Additionally, we show that overexpression of *trim25* leads to protection from ISAV infection. Utilizing RNA sequencing we show that knock-out of *trim25* reduces expression, and highlights the mechanistic role of TRIM25 in the innate immune response. This research highlights the key antiviral role of *trim25* in fish, and paves the way for biotechnological applications to reduce the impact of viral outbreaks in salmon aquaculture.

**Keywords**: CRISPR, Gene editing, ISAV, Atlantic Salmon

# Session 2 — Immunity, stress & welfare II

## **Chairs:** Robert Stewart, Jerko Hrabar

### EARLY-LIFE CONDITIONING INFLUENCES LATER-LIFE STRESS RESPONSES IN RAINBOW TROUT: INSIGHTS FROM MULTI-OMICS ANALYSES

Shankregowda, A.M.[1], Overland, B.[1], Hotz, A.L.[2], Qi, W.[2], Hitchings, M.[3], Uren Webster, T.M.[1], Garcia de Leaniz, C.[1,4], Ralph Schlapbach[2], Fernandes, J.M.O.[5] and Consuegra, S.[1,6]

*[1]Department of Biosciences, Swansea University, Swansea, Wales, UK, [2]Functional Genomics Center Zurich (FGCZ), University of Zürich, Zürich, Switzerland, [3]Institute of Life Science, Swansea University, Swansea, Wales, UK, [4]Centro de Investigaciones Marinas, Universidade de Vigo, Vigo, Spain, [5]Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain, [6]Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain*

e-mail: a.m-shankregowda@swansea.ac.uk

In aquaculture, fish normally encounter stressors such as temperature fluctuations, handling and crowding stress. Early developmental stages represent a critical period during which environmental conditions can shape long-term physiological responses through developmental plasticity. Early-life stress exposure may therefore induce persistent molecular changes mediated by epigenetic mechanisms, including regulatory small RNAs and DNA methylation, which can influence gene expression and host–microbiome interactions. In this study, we investigated whether early-life stress conditioning during embryonic development influences later-life stress responses in rainbow trout (*Oncorhynchus mykiss*). Embryos were exposed to a controlled early-life stress conditioning (cold shock) and at a later stage fish were subjected to density stress, to evaluate whether early-life conditioning alters their response to a secondary stress challenge. Long-term effects were assessed using an integrated multi-omics approach, including gut microbiome profiling, miRNA expression, transcriptome and methylation analysis. Significant differences were observed in the gut microbiome composition, including shifts in both alpha and beta diversity. Early-life conditioned fish exposed to density stress showed reduced transcriptional response compared with fish exposed only to density stress, suggesting a modified stress response. miRNA profiling by small RNA-sequencing identified 294 differentially expressed miRNAs in early-life conditioned fish exposed to density stress (acute). Several of these miRNAs are associated with neurodevelopmental stress signalling, immune system programming, development and metabolic adaptation. Next, we will compare differential methylation levels between early-conditioned and non-conditioned fish. Overall, our initial results indicate that early developmental stress can influence later-life physiological responses and host–microbiome interactions, highlighting the importance of early-life stress conditioning and epigenetic mechanisms in shaping stress resilience in aquaculture species.

**Keywords**: Stress, Early-life conditioning, Multi-omics, Epigenetics

# THE EMBRYONIC ENVIRONMENT: KEY TO ROBUSTNESS IN ATLANTIC SALMON

Burgerhout, E.[1], Milton, C.[2], Lazado, C.C.[1], Tengs, T.[1], Wade, N.[2], Fernandes, J.M.O.,[3] Macqueen, D.J. [2] and Henkel, C.V.[4]

*[1]Nofima, Tromsø, Norway;[2] The Roslin Institute, University of Edinburgh, Scotland; [3]Institute of Marine Sciences, Barcelona, Spain; [4]NMBU, Ås, Norway.*

e-mail: erik.burgerhout@nofima.no

During the last two decades, the potential impact of the embryonic environment to improve aquaculture performance later in life received more attention, yet many questions remain to be unraveled. Most interest has been pointed towards embryonic temperature, which regulates a vast array of physiological and biochemical processes, including the speed of development. In Atlantic salmon, embryonic temperature has been linked to heart and skeletal deformities, as well as muscle development and growth later in life. Considering the high mortalities in the industry, largely due to complex infectious disease landscapes and potentially suboptimal organ development, we hypothesised that embryonic temperature affects the functional development of the immune system and disease resistance later in life.

From fertilisation until start feeding, embryos were exposed to different temperature regimes; thereafter the same cohort was given the same temperature across the rest of ontogeny.

Juveniles (~20g parr) were challenged with *Yersinia ruckeri*, a bacterium that causes enteric redmouth disease. Survival was higher in groups exposed to lower embryonic temperatures ($4^0$C) compared to those exposed to $8^0$C. A time-line focusing on the short- and long-term dynamics of the response to *Y. ruckeri* in head kidney, a primary hematopoietic and immune tissue, was investigated using RNA-seq and single nucleus RNAseq (snRNA-seq). Our results show strong evidence for both constitutive and infection-responsive differences between the embryonic temperature groups, affecting some immune cell types more than others.

Using snRNA-seq we also aim to create a cell atlas of early ontogeny, which will be deployed to explore the impact of the environment on organ development, specifically heart, gills and kidney.

Understanding the developmental pathways and molecular mechanisms resulting in long-term phenotypic programming could be exploited by the industry to enhance salmon health and welfare.

**Keywords:** Embryogenesis, Immunity, Transcriptomics, Bacterial challenge

# IDENTIFICATION OF FISH EPIGENETIC BIOMARKERS OF DISEASE RESISTANCE DRIVEN BY EARLY REARING CONDITIONS

Huang, W.[1], Friis, E.[1], Hitchings, M.[4], Uren Webster, T.M.[1], Thorland, I.[3], Fannemel, B.[3], Moghadam, H.K.[3,4], Houston, R.[3] and Consuegra, S.[1,2]

*[1]Department of Biosciences, Centre for Sustainable Aquatic Research, Swansea University, UK, [2]Instituto de Investigaciones Marinas, IIM-CSIC Vigo, Spain, [3]Benchmark Genetics As, Brandbenken 1, 5003 Bergen, Norway, [4]TYR, Storhamargata 44 N-2317 Hamar, Norway*

e-mail: s.consuegra@swansea.ac.uk; sconsuegra@iim.csic.es

Understanding the basic process of fish adaptation to the farming environment (domestication) is critical to improving production and preventing negative consequences of wild-farm fish interactions. We are investigating non-genetic mechanisms (epigenetic) influenced by the rearing environment (temperature, diet, light conditions, population density) which can help fish to adapt to the captive environment quickly. These include epigenetic changes associated with early rearing conditions (temperature) with influence on resistance to disease. For this, embryos from nine different Atlantic salmon families were incubated at two different temperatures to the alevin stage (potentially changing the gene regulation through epigenetic mechanisms), after which both groups were reared at the same temperature. Juveniles were challenged with a pathogen common in salmon farms (Infectious Salmon Anemia Virus (ISA)), ensuring that the results are fully relevant for the commercial process. Families incubated at different temperatures displayed different survival to ISAV. Juvenile heart samples from live and dead fish incubated at the two temperatures were analysed for DNA methylation differences using ONT. Our previous evidence indicated that fish subject to different temperatures at early rearing display epigenetic changes and different response to stress in terms of gene expression, suggesting that these changes could result in different responses to disease. We investigated differentially methylated regions (DMRs) by applying the stringent criteria to ensure high-confidence results: each region was required to contain at least five cytosines and span a minimum of 100 base pairs. Regions were considered differentially methylated only if the average methylation difference between conditions was at least 20% and all constituent cytosines showed a statistically significant difference with a p-value ≤ 0.001. We identified 1,207 DMRs, including 701 DMRs within gene bodies of 658 genes and 132 DMRs within putative promoter regions of 128 genes (defined as 2,000 bp upstream of the transcription start site). A significant GO term (Proline metabolic process, potentially related to energy production and stress response) was identified for the 128 genes containing DMRs within putative promoter regions, and KEGG pathway analysis included Cell adhesion molecule (CAM) interaction and MAPK signalling pathway, potentially involved in immune response, apoptosis, and inflammation. These results indicate that early rearing temperature affects salmon DNA methylation in genes possibly related to disease response, and we are currently identifying particular epigenetic markers related to ISAV survival.

**Keywords**: Early life conditioning, Methylation, Nanopore sequencing, Atlantic salmon, ISAV

# CHALLENGES AND CONSIDERATIONS FOR NANOPORE DNA METHYLATION SEQUENCING IN ATLANTIC SALMON

Friis, E.[1], Huang, W.[1], Hitchings, M.[3], Uren Webster, T.M.[1], Thorland, I.[4], Fannemel, B.[4], Moghadam, H.K.[4], Houston, R.[4], and Consuegra, S.[1,2]

*[1]Department of Biosciences, Centre for Sustainable Aquatic Research, Swansea University, UK, [2]Instituto de Investigaciones Marinas, IIM-CSIC Vigo, Spain, [3]TYR, Storhamargata 44 N-2317 Hamar, Norway, [4]Benchmark Genetics As, Brandbenken 1, 5003 Bergen, Norway*

e-mail: e.m.friis@swansea.ac.uk

Nanopore sequencing has become an increasingly attractive platform for DNA methylation analysis due to its ability to detect base modifications directly from native DNA. However, its application to large and complex genomes presents technical challenges. In addition to whole-genome sequencing (WGS), nanopore platforms enable adaptive sampling (AS), a real-time enrichment approach in which DNA molecules are selectively sequenced or rejected based on rapid alignment of the initial portion of each read to a pre-determined set of target regions. Here, we ran a small-scale trial comparing WGS and AS on Atlantic salmon genomic DNA using Oxford Nanopore Technologies (ONT). Adaptive sampling was implemented to selectively enrich putative promoter regions, defined as 3,000bp upstream and downstream of transcription start sites (TSS), in order to increase coverage of regulatory regions relevant for differential methylation analysis. While adaptive sampling provides a flexible strategy for targeted sequencing without additional library preparation, its performance can be influenced by genome characteristics such as GC-rich and highly repetitive regions. Across both WGS and AS sequencing runs, we observed a rapid decline in active nanopores, resulting in reduced sequencing coverage compared with typical runs using genomic DNA from other organisms. Adaptive sampling achieved some enrichment of the targeted promoter regions, but the level of enrichment did not improve overall coverage beyond that achieved by WGS. Reduced coverage in both strategies may be due to secondary structures within salmon DNA or compounds that co-purify during DNA extraction, both of which could increase pore blockage events during sequencing, although the exact cause remains to be determined. Our trial of AS and WGS using genomic Salmon DNA highlights some limitations of nanopore sequencing for large, repetitive genomes. Certain organisms, including salmon, appear to cause pore blockages that reduce overall sequencing performance. While optimised library preparation and careful sample QC can modestly improve coverage and yield, they do not fully overcome these challenges. These findings suggest that nanopore sequencing may not always be the ideal platform for sequencing such genomes, and careful consideration of library preparation and sequencing platform is essential.

**Keywords:** Nanopore sequencing, DNA methylation, Adaptive sampling, Atlantic salmon, Pore blockage

# GENOMIC EVIDENCE FOR CONSERVED ENVIRONMENTAL SENSING REGULATORS UNDERLYING DOMESTICATION AND BREEDING-RELATED DIVERGENCE IN GILTHEAD SEABREAM (*SPARUS AURATA*)

Moulistanos, A.[1,2,4], Mitsis, A.[1], Gkagkavouzis, K.[1,2], Karaiskou, N.[1,2], Antonopoulou, E.[1], Triantafyllidis, A.[1,2], Ahi, E.P.[3] and Papakostas, S.[4]

*[1]Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece, [3]University of Helsinki, Helsinki, Finland, [4]International Hellenic University, Thessaloniki, Greece*

e-mail: amoulist@bio.auth.gr

Domestication in fish involves rapid and complex changes in life-history, physiology, and behavior under human-controlled conditions. In gilthead seabream (*Sparus aurata*), domestication and selective breeding programs have generated measurable genetic divergence from wild populations. However, the genomic mechanisms linking domestication to production-relevant traits remain incompletely understood. We analysed genome-wide Pool-Seq data from 10 farmed and 10 wild seabream populations from six countries across the Mediterranean. We investigated 5.3 million SNPs and identified genomic regions associated with domestication and breeding-related divergence, particularly on chromosome 19, involving genes associated with environmental sensing mechanisms that regulate stress, endocrine, immune, and reproductive responses critical for performance under aquaculture conditions. Prominent candidate genes include *ahrra*, a regulator of the ancient AHR–ARNT/HIF environmental-sensing pathway, *kdm6al*, a chromatin modifier that coordinates developmental and stress responses, and *pigm*, a gene involved in GPI-anchor biosynthesis that affects cell-surface composition and host defence. These regulatory systems are deeply conserved across animals, suggesting that domestication and selective breeding may involve modifications of conserved environmental sensing and regulatory networks that influence key physiological and reproductive traits. Taken together, our results identify conserved molecular pathways and candidate genes that may support gene-informed breeding strategies to improve stress resilience and reproductive performance in aquaculture.

**Keywords**: artificial selection; genome-wide divergence; environmental sensing mechanisms; rapid adaptation; marine teleost

# IDENTIFICATION OF NEW KEY STRESS RELATED GENES BY COMPARATIVE ANALYSIS OF THE GENE EXPRESSION PROFILE UNDER MULTIPLE ENVIRONMENTAL STRESSORS IN GILTHEAD SEABREAM (*SPARUS AURATA*)

Silva, I.A.L. [1*], Gonçalves-Martins, J.P. [1*], Angelo, M.[1], Pousão-Ferreira, P.[1,2] and Lourenço-Marques, C.[1,2]

[1] *S2AQUA, Olhão, Portugal,* [2] *IPMA/EPPO, Olhão, Portugal; *Contributed equally.*

e-mail: iris.silva@s2aquacolab.pt

In aquaculture, stress is a major concern, as it significantly affects fish physiology, welfare, and productivity. Several methodologies have been developed to assess stress levels, including the use of molecular biomarkers such as *hsp70*, *hsp90*, and *metallothioneins*. However, despite their potential, these biomarkers are often associated with high variability and limited reliability, leading to possible misinterpretation of stress responses. To address this limitation, this study applies a transcriptomic approach to identify novel molecular biomarkers of stress in gilthead seabream (*Sparus aurata*). Fish were subjected to two acute stress conditions, ammonia and hypoxia exposure, at two different developmental stages (70 and 90 DAE), and sampled at multiple time points (0.5 h, 1 h, 3 h and 6 h) to capture the temporal dynamics of gene expression. The results revealed distinct gene expression profiles between the control group (non-stressed fish) and both stress conditions, demonstrating that stress indeed influenced gene expression patterns. Several DEGs were identified as candidates for novel stress-specific biomarkers, such as *dusp5* and *slc25a48*, which were only present when exposed to ammonia stress in both developmental stages. In hypoxia, no genes were 100% exclusive, some were present after hypoxia exposure but also in one developmental stage after ammonia exposure, such as *klhl33* and *celsr2*. A subset of genes was consistently differentially expressed across all conditions, suggesting their potential as general-stress biomarkers, including *klhl38b*, *fosl1* and *nr4a3*. Traditional stress biomarkers, such as heat-shock proteins and metallothioneins, were differentially expressed but showed lower fold changes and inconsistent expression patterns across conditions and developmental stages compared to the identified candidate genes. Temporal analysis revealed different gene expression profiles between early and late transcriptional responses, highlighting the importance of sampling time in stress biomarker identification and future use. Selected candidate genes were further validated using quantitative PCR. This approach seeks to improve the accuracy and robustness of stress assessment in aquaculture by identifying more consistent and reliable molecular biomarkers, ultimately contributing to better monitoring of fish welfare and production conditions.

**Keywords**: Gene expression, stress, molecular biomarkers

**Acknowledgments:** This study had the support of the project Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148).

# CIRCADIAN REGULATION OF IMMUNE FUNCTIONS IN ATLANTIC SALMON LEUKOCYTES

Lazado, C.C. [1], Wasmuth, M. [1], Hansen, M.H. [1], Burgerhout, E. [1], Tengs, T.[1], Andersen, Ø. [1] and Sundaram, A.Y.M. [2]

[1]*Nofima, Tromsø, Norway;* [2]*Dept. Of Medical Genetics, Oslo University Hospital, Oslo, Norway.*

e-mail: carlo.lazado@nofima.no

Circadian rhythms are endogenous, approximately 24-hour cycles that regulate a wide range of physiological and cellular processes in vertebrates, including immune function. However, knowledge of circadian regulation in fish immune cells, particularly head kidney leukocytes (HKLs), remains limited. Using Atlantic salmon HKLs, we aimed to address the following questions: 1) *Do HKLs express key regulators of the circadian clock, and does their global transcriptome exhibit circadian rhythmicity?* 2) *Do HKLs respond to the pathogen-associated molecular pattern lipopolysaccharide (LPS) in a time-dependent manner?* 3) *Does inhibition of clock function abolish time-dependent immune responses in HKLs?*

Atlantic salmon HKLs express the major components of the vertebrate circadian clock, including *clock*, *period*, and *cryptochrome* genes. Under a 12 h light:12 h dark (12L:12D) photoperiod, daily changes in gene expression were maintained *in vitro* for several days. Five circadian models were applied to analyse transcriptome-wide rhythmicity following five days of entrainment under either 12L:12D or constant darkness (0L:24D). Approximately 64% of the transcriptome did not exhibit rhythmicity *in vitro*. Notably, around 36% of transcripts displayed rhythmic expression under both photoperiod conditions, and approximately 25% of these maintained rhythmicity in the absence of a light cue. Among rhythmic genes, changes in timing and/or amplitude were more common than complete loss of rhythmicity. In addition, changes in mean expression levels frequently co-occurred with rhythmic alterations. Distinct time-dependent transcriptomic responses were observed when HKLs entrained to 12L:12D were challenged with LPS at ZT4 (day) or ZT16 (night). Clock genes responded in a temporal manner: *clock* and *per1* were downregulated following LPS challenge at ZT4, whereas *cry3b* was downregulated at ZT16. Functional immune responses also varied by time of challenge. Myeloperoxidase production and respiratory burst activity were higher at ZT4, while phagocytic activity was elevated at ZT16. Differential expression analysis identified 16,124 significantly affected genes following LPS challenge at ZT4, compared with 10,567 at ZT16. We are currently investigating how loss of clock function, achieved through CRISPR-Cas9–mediated gene knockout, affects leukocyte immune function. These data will be presented at the symposium.

**Keywords**: CRISPR, Immune cells, Immunity, Molecular clocks, RNA-Seq

# SEX-DEPENDENT REGULATION OF IMMUNE RESPONSES IN EUROPEAN SEA BASS GONADS

López-Chillarón, S. [1,2], Caballero-Huertas, M. [3,4], Casals, E. [5,6], Esteve-Codina, A. [5,6] and Ribas, L. [1]

*[1]Institute of Marine Sciences, Barcelona, Spain; [2]Autonomous University of Barcelona, Bellaterra, Spain; [3]CIRAD, Montpellier, France; [4]ISEM, Montpellier, France; [5]National Center for Genomic Analysis, Barcelona, Spain; [6]University of Barcelona, Barcelona, Spain*

e-mail: sandralopez@icm.csic.es

European sea bass is one of the most economically important species in European aquaculture. In the present study, we investigated sex-specific molecular mechanisms underlying the immune response in gonadal tissues 48 h after a bacterial challenge by using sequencing approaches. DNA methylation patterns were analysed using the Enzymatic Methyl-seq (EM-seq) method. Then, differentially methylated genes (DMGs) were identified and compared with differentially expressed genes (DEGs) dataset available, and the selection of immune-related candidates as potential sex-specific biomarkers was performed and analysed by qPCR. Our results revealed sexual dimorphism between the gonadal tissues, with ovaries showing 163,205 methylated cytosine sites (CpGs), and testes showing 103,588 caused by the bacterial infection. Furthermore, ovaries showed a predominance of hypermethylated CpGs, whereas testes exhibited more hypomethylated ones. The overlap of those genes that were differentially methylated and showed changes in their gene expression at the same time was stronger in testes (700 DMGs and DEGs) than in ovaries (60 DMGs and DEGs). Notably, several immune-related genes exhibited sexually dimorphic DNA methylation patterns, with opposite CpG methylation profiles between ovaries and testes or restricted to a single gonadal tissue. Sexual dimorphism was present in non-infected gonads, showing in ovaries a significant hypomethylation in all selected immune genes and a down-regulation tendency in their expressions. After the bacterial challenge, the ovaries showed upregulation or no changes in these genes, whereas in the testes, the tendency was towards downregulation. These results highlight distinct molecular regulatory mechanisms of immune genes between ovaries and testes in response to bacterial infection, supporting a sex-dependent epigenetic modulation of gene expression in the immune response of European sea bass.

**Keywords**: DNA methylation, gene expression, sexual dimorphism, immune response, European sea bass

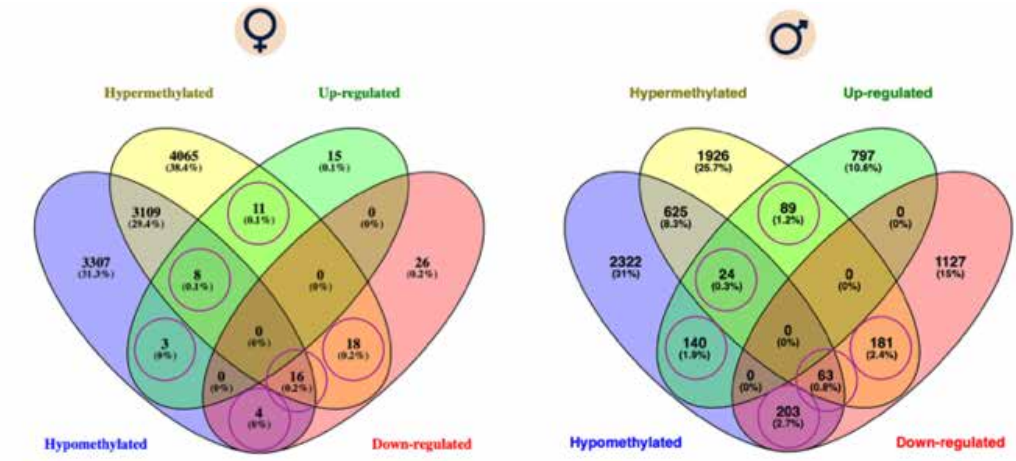



***Figure 1.*** *Overlapping between differentially methylated genes (DMGs) and differentially expressed genes (DEGs) in European sea bass gonads after a bacterial challenge*

# DAY 2 — Tuesday, 5 May 2026

## Session 3 — Immunity, stress & welfare III

**Chairs:** Sofia Consuegra, Tim Regan

### DECODING THE GENETIC BASIS OF FISH IMMUNE RESPONSES USING KNOCKOUT CELL LINES

Veiga-Rúa, S. [1], Gallo, M. [1], Pérez-Pereira, N. [1], Rodríguez-Vázquez, R. [1], Rodríguez-Fernández, M. [1], Torres-Sedano, M. A. [1], Suárez-Rivas, A. [1], González-Sánchez, A. [1], Cuesta, A. [2], Robledo, D. [1,3]

*[1]Department of Zoology, Genetics and Physical Anthropology, Faculty of Biology, University of Santiago de Compostela, Santiago de Compostela, Spain; [2]Immunobiology for Aquaculture Group, Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, Murcia, Spain; [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Midlothian, United Kingdom.*

e-mail: saraveiga.rua@usc.es

Understanding the molecular pathways involved in disease resistance is essential for improving survival of farmed fish. However, the mechanisms underlying fish immune responses remain largely uncharacterized. In this context, genome-edited fish cell lines are emerging as powerful models to disentangle these and other complex traits. Despite this potential, there are few available methods to efficiently modify fish genomes. Here, we present optimized protocols for delivering nucleic acids and CRISPR/Cas9 ribonucleoproteins into three aquaculture-relevant fish cell lines: SaB-1 (Sparus aurata), DLB-1 (Dicentrarchus labrax), and CHSE-214 (Oncorhynchus tshawytscha).

As a proof of concept, we knocked-out irf3 (interferon refulatory factor 3) in seabream and seabass cells. IRF3 is a transcription factor involved in the regulation of the innate immune signalling pathways, playing an important role in the activation of type I interferon. To further characterize the immune response landscape in fish, we also investigated several TRIM (Tripartite Motif) E3 ubiquitin ligases. This protein family shows a rapid evolution and remarkable diversification across species, suggesting important and potentially specialized functions in antiviral defence and immune regulation. IRF3 and TRIM knockout models were stimulated with poly I:C to mimic viral infection before assessing alterations in the antiviral response by RT-qPCR, Western blot and RNA-seq.

Together, these optimized genome-editing workflows and knockout models will help dissecting antiviral pathways in aquaculture species, thus paving the way for deeper functional insights and the development of more resilient farmed fish.

**Keywords**: CRISPR/Cas9, in vitro, immune system, TRIM, IRF3

# HOW COULD GENOMIC KNOWLEDGE HELP MAKE ATLANTIC SALMON MORE "COHO-LIKE" IN THEIR ABILITY TO RESIST SEA LICE?

Robinson N.A. [12], Sveen L. [1], Aslam L. [1], Østbye T-K.K. [1], Gjerde B. [1], Krasnov A. [1], Dagnachew B.S. [1], Lillehammer M. [1], Tengs T. [1], Wasmuth M. [1], Vaadal M. [1], Gonen S. [3], Kristjánsson Ó.H. [3], Thorland I. [3], Houston R. [3], Rye M. [3], Fast M.D.[4], Semple, S.L. [4], Baranski M. [5], Gulliksen S. [5], Boison S. [5], Salisbury S. [6], Kurian D. [6], Øvergård A.C. [7], Doherty Midtbø H.M. [7], Wargelius A. [8], Bizuayehu T.T. [8], Kjærner-Semb E. [8], Edvardsen R.B. [8], Bron J. [9], Monaghan S. [9], Daniels R.R. [9], McGowan M. [9] and Robledo D. [6]

*[1]Nofima AS, Tromso, Norway, [2]Deakin Marine, Deakin University, Australia, [3]Benchmark Genetics, Milton Bridge, Penicuik, UK, [4]Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada, [5]Mowi AS, Bergen, Norway, [6]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK, [7]University of Bergen, Bergen, Norway, [8]Havforskningsinstituttet, Bergen, Norway, [9]Institute of Aquaculture, University of Stirling, Stirling, UK*

e-mail: nicholas.robinson@nofima.no

Sea lice infection and removal are of great animal welfare and economic concern to Atlantic salmon farming around the world. Pacific species of salmon (such as pink and coho) have very effective natural immune defences against sea lice. We have applied the latest genomic technologies, and CRISPR gene editing, to gain a better understanding of the genetic and cellular mechanisms used by Pacific salmon species to fight sea lice. But how might we utilise this knowledge effectively to reduce lice infection and the need for delousing by Atlantic salmon aquaculture industries? Here we discuss and compare two potential opportunities to utilise such knowledge. We consider possibilities for measuring and utilising the genomic and cellular immune response as a phenotype for selective breeding and potential future opportunities for the creation and dissemination of gene edited fish. Societal, technical and practical challenges with both approaches will be discussed.

**Keywords:** snRNAseq, spatial transcriptomics, CRISPR, sea lice resistance

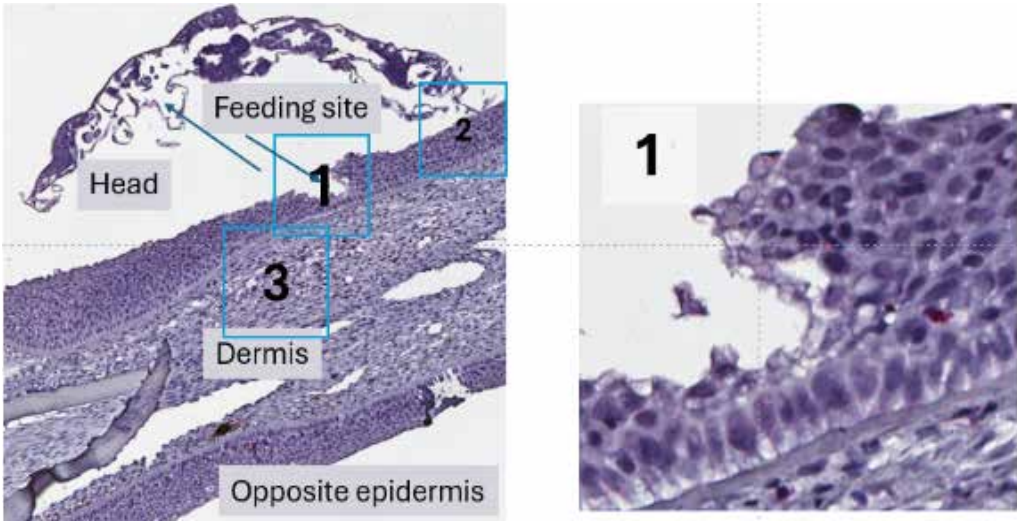



***Figure 1.*** *Immune cell marking using RNAscope could provide direct immune response phenotypes to inform selective breeding. A few immune cells present in the Atlantic salmon skin at the lice feeding site are stained in red (at position 1 in the Atlantic salmon skin directly under the lice).*

# EPITRANSCRIPTOMIC REGULATION OF ATLANTIC SALMON DURING *CALIGUS ROGERCRESSEYI* INFESTATION

Valenzuela-Muñoz, V. [1], Gallardo-Escárate, C.[1], Valenzuela-Miranda, D.[1] and Roberts, S.[2]

*[1]Interdisciplinary Center for Aquaculture Research (INCAR2), University of Concepción, Concepción, Chile; [2]School of Aquatic and Fishery Sciences, University Of Washington, Seattle, USA*

e-mail: valevalenzuela@udec.cl

The molecular mechanisms underlying parasite resistance in marine species have been widely investigated using transcriptomic analyses and genome-wide association studies. However, RNA-level epigenetic regulation during host–pathogen interactions remain largely unexplored in aquaculture genomics. This study evaluated mRNA modifications in Atlantic salmon during infestation by the sea louse *Caligus rogercresseyi*. Resistant (R) and susceptible (S) Atlantic salmon families were analyzed during parasitism using skin tissue samples. Molecular data were generated using Illumina RNA sequencing and nanopore direct RNA sequencing (DRS), and reads were mapped to the Atlantic salmon genome. Transcriptomic analyses revealed strong modulation of genes associated with immune responses, including immunoglobulins, major histocompatibility complex class I (MHC I), and Toll-like receptors (TLRs), as well as genes involved in tissue repair such as collagen and keratin. DRS analysis further identified putative epitranscriptomic modifications in genes associated with both phenotypes, including hemoglobin, cathepsin, cathelicidin, ferritin, and collagen genes. These findings provide the first evidence of RNA methylation marks associated with resistance and susceptibility phenotypes in Atlantic salmon during sea lice infestation, highlighting a potential role for epitranscriptomic regulation in host responses to parasitic infection.

**Keywords**: Epitranscriptome, Atlantic salmon, sea lice, resistant/susceptible families.

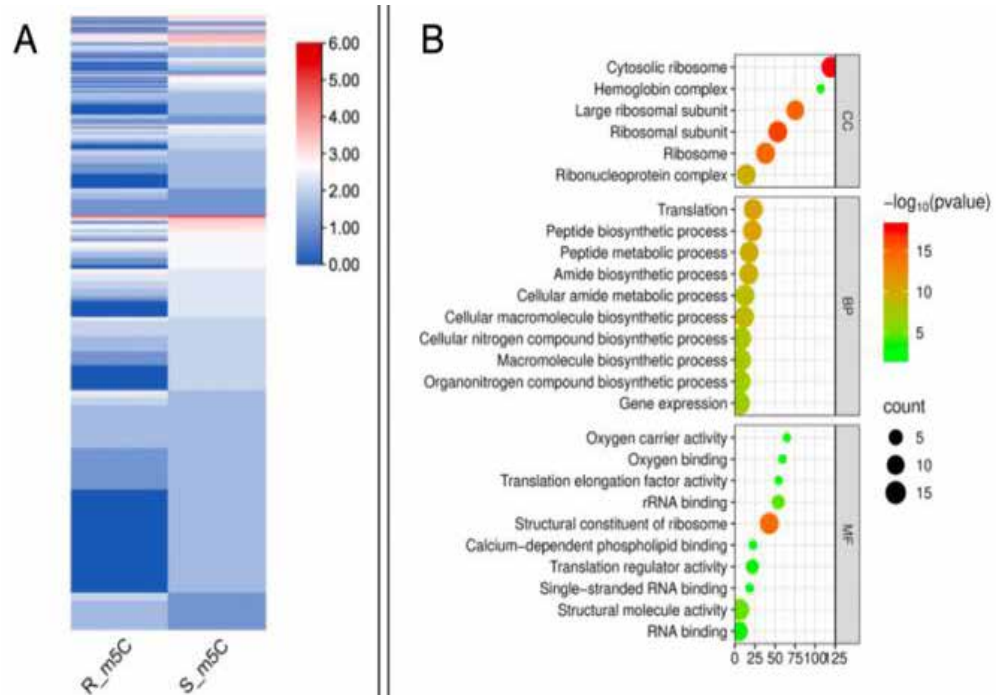



***Figure 1.*** *mRNA with m5C modification in resistant and susceptible Atlantic salmon phenotypes infected with sea lice.*

**Funding:** *ANID INCAR2 CIA250009*

# DIFFERENTIAL GILL TRANSCRIPTOME RESPONSES IN ATLANTIC SALMON TO CLONAL AND REISOLATED CULTURES OF *NEOPARAMOEBA PERURANS*, AGENT OF AMOEBIC GILL DISEASE

Iversen, M.[1], Andersen, L.[2], Brenne, H.[1], Sundaram, A.Y.M. [3] and Lazado, C.C.[4]

[1]*Nofima, Tromsø, Norway; Norway;* [2]*ILAB, Bergen, Norway;* [3]*Norwegian Sequencing Centre, Oslo, Norway;* [4]*Nofima, Ås, Norway*

e-mail: marianne.iversen@nofima.no

The gill is a vital multifunctional organ in fish, responsible for gas exchange, ion regulation, acid-base balance, and ammonia excretion. As a mucosal organ and an active interface between the external and internal environment, it also plays an important role in immunity. Gill health issues are among the main challenges facing salmon aquaculture, where they have a considerable impact on fish welfare, health and survival. Amoebic gill disease (AGD), caused by *Neoparamoeba perurans*, is a significant gill disease affecting Atlantic salmon during the seawater phase of production and is commonly detected at Norwegian marine aquaculture sites, particularly during late summer and early autumn. We exposed Atlantic salmon recently transferred to salt water to clonal and reisolated cultures of *N. perurans*. Infection with the clonal cultures was performed at two different concentrations. Gill scores and samples for RNA-Seq were taken at 21 days post-infection. AGD presence was controlled by taking mucosal gill swabs for targeted qPCR. Gill scores indicate a lower level of virulence by the clonal cultures. Analysis for differentially expressed genes (DEGs) between the groups show minimal difference between the two groups infected by clonal *N. peruans*, while both groups show a partially overlapping differential response when compared to the group infected by reisolated *N. peruans*. Focused analysis of key genes previously linked to AGD infection revealed significant differences in expression between treatment groups. These findings indicate that genetic diversity of the amoebic culture used in infection studies influence i) virulence and ii) host response.

**Keywords:** RNA-Seq, Atlantic salmon, gill disease, AGD, host response

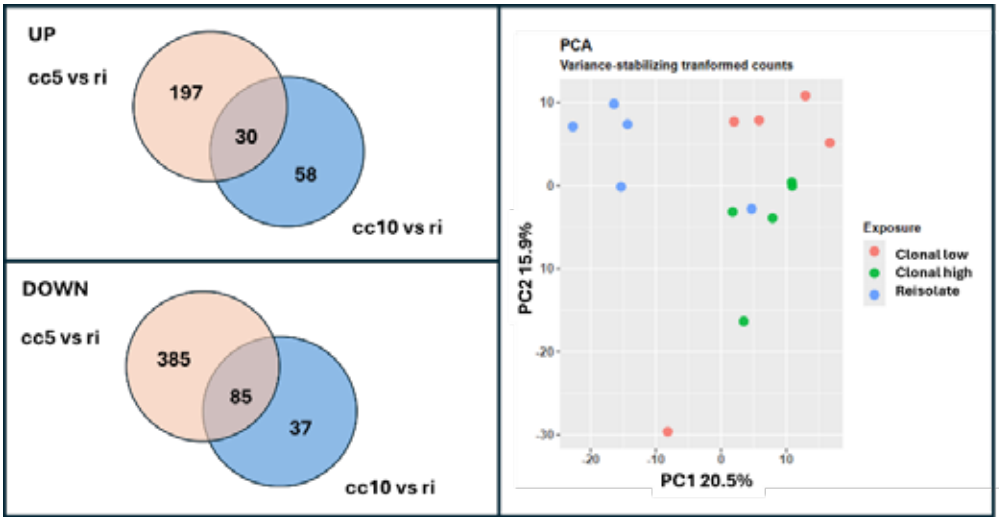



***Figure.1*** *Left panes showing Venn diagrams illustrating the number of similarly behaving differentially expressed genes when contrasting clonal and polyclonal treatments. Right pane is a PCA plot showing the clustering of the three treatments.*

# CIRCULATING MICRORNAS AS EMERGING BIOMARKERS FOR STRESS MONITORING IN *OCTOPUS VULGARIS* AQUACULTURE

Pereira, M.[1], Castro-Iglesias, O.[1], Guerrero-Peña, L.[1], García-Fernández, P.[2], Touriñan, P.[2], Chabarrias, D.[2], Tur, R.[3], Rubiolo, J.A.[4], Saura, M.[1] and Rotllant, J.[1]

*[1]Aquatic Biotechnology Lab. Instituto Investigacións Marinas (IIM-CSIC), Vigo, Spain; [2]Pescanova Biomarine Center, O Grove, Pontevedra, Spain; [3]Green Parrot Aquatic Solutions. S.L. Madrid. Spain; [4]Universidad de Santiago de Compostela, Lugo, Spain*

e-mail: mpereira@iim.csic.es

The common octopus (*Octopus vulgaris*) possesses significant socio-economic value. However, the recent decline in wild captures has raised concerns within the fishing sector, leading to the consideration of aquaculture as an alternative. A major hurdle in this industrial transition is the guarantee of animal welfare, which currently lacks reliable objective measurement tools. Traditional endocrine biomarkers used in vertebrates, such as cortisol and corticosterone, are not involved in the stress response of octopuses, creating a critical gap in welfare monitoring. MicroRNAs (miRNAs), particularly circulating forms, represent promising non-invasive biomarkers due to their regulatory roles and stability in body fluids. This study aimed to characterize circulating miRNAs in hemolymph under hyposalinity stress, a common environmental stressor for this species. miRNA detection was evaluated across hemolymph fractions (whole hemolymph, plasma, and cellular components). A total of 119 known miRNAs were identified, along with 189 newly predicted candidates. Among known miRNAs, 25 showed differential expression under osmotic stress, while two novel candidates were proposed as potential regulators involved in osmoregulatory responses. These results reveal circulating miRNAs potentially involved in osmotic stress adaptation and provide genomic resources supporting the development of non-invasive biomarkers and improved physiological monitoring in *O. vulgaris* aquaculture.

**Keywords**: microRNAs, octopus, biomarkers, welfare assessment

**Acknowledgements:** This research was funded by the MCIN/AEI/10.13039/501100011033 grant number PID2024-161686OB-100 and CSIC intramural project PIE-202340E076 to JR and by “ERDF A way of making Europe”

# UNCOVERING THE MICRORNA REGULATORY LANDSCAPE OF TURBOT

Aramburu, O. [1,2], Blanco-Hortas, A. [1,2], Pardo, B. G. [1], Bobé, J. [3], Bouza, C. [1] and Martínez, P. [1]

*[1] Dept. Zoology, Genetics and Physical Anthropology, Campus Terra, Universidade de Santiago de Compostela (USC), Lugo, Spain. [2] GENEAQUA S.L., Lugo, Spain. [3] National Insitute of Agriculture, Food and Environment (INRAE), Rennes, France*

e-mail: oscar.aramburu@geneaqua.com

MicroRNAs (miRNAs) are key post-transcriptional regulators that shape gene expression during development, tissue differentiation, and physiological responses. However, comprehensive miRNA resources remain limited for many aquaculture species. Here, we present a genome-wide characterization of the turbot (*Scophthalmus maximus*) miRNA repertoire and its regulatory landscape across development, tissues, and immune conditions. Using 174 high-quality sequencing libraries (115 mRNA-seq and 59 small RNA-seq), this work profiles embryos spanning major developmental stages, multiple tissues, and head kidney samples following mimics of bacterial and viral challenges.

We have identified over 300 miRNA genes producing more than 450 mature miRNAs. A small core set dominated overall expression, accounting for a large fraction of total miRNA abundance. Condition-specificity analysis identified over 100 miRNAs with highly specific expression ($\tau \geq 0.9$), most prominently in brain, followed by late developmental stages and immune tissues (Figure 1). These patterns reflected conserved biological roles, including neuronal regulation (miR-9, let-7), immune responses (miR-146), liver metabolism (miR-122), and muscle differentiation (miR-1/206).

Target prediction identified more than 20,000 potential miRNA–mRNA interactions, and above suggestive/putative 7,000 targets actively expressed. Differential expression analyses revealed extensive regulatory changes mainly across embryogenesis and between ovaries and testis in the adults.

**Keywords**: microRNA, turbot, transcriptomics, regulatory networks, genomic

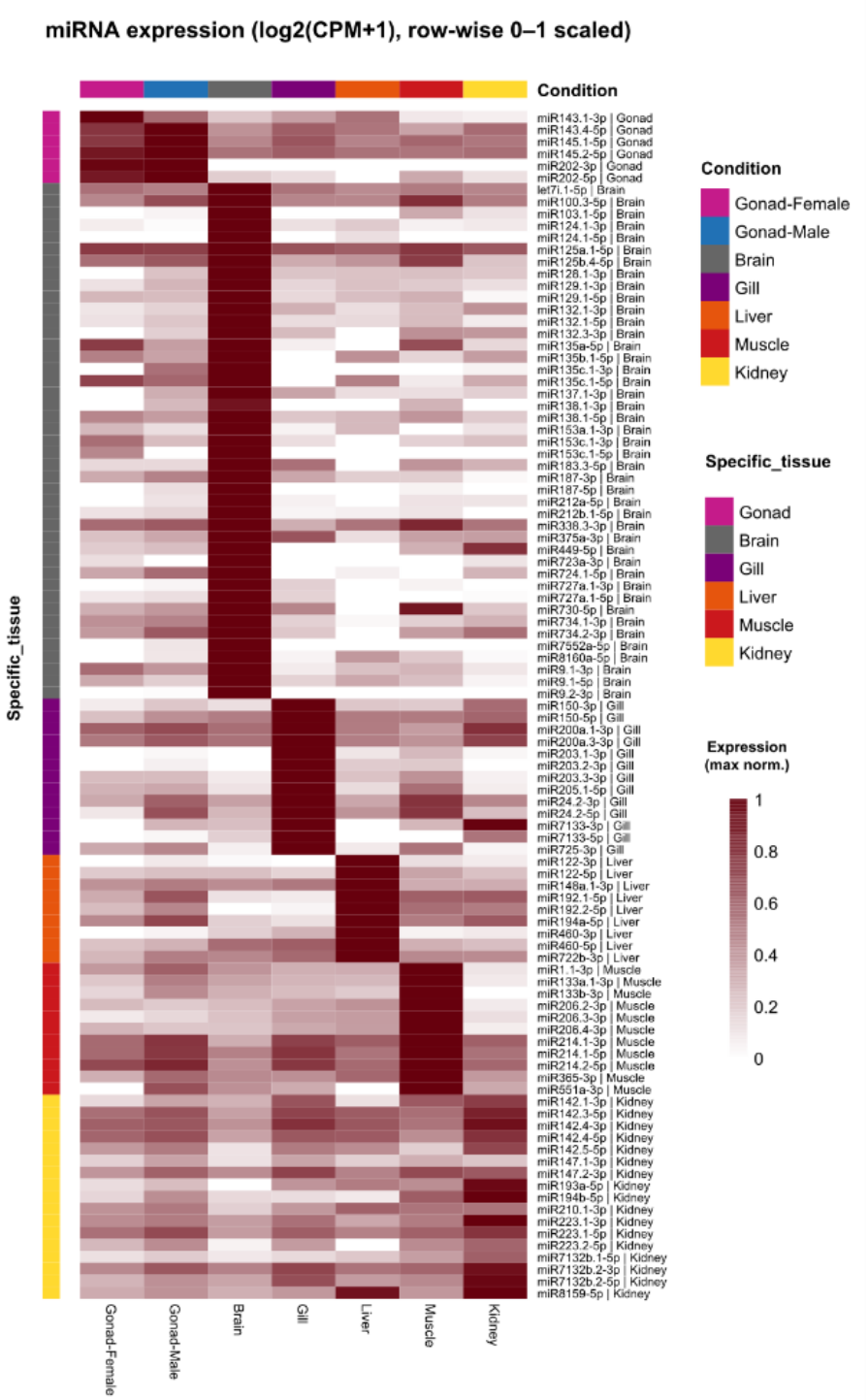



***Figure 1.*** *Average normalized expression of selected condition-specific miRNAs ($\tau \geq 0.9$), across selected conditions.*

## Session 4 — Nutrition & growth

## **Chairs:** Jorge Fernandes, Ivana Lepen Pleić

### DIET SHAPES FUNCTIONAL HOST–MICROBIOTA ASSOCIATIONS IN GILTHEAD SEABREAM REVEALED BY INTEGRATED TRANSCRIPTOME AND MICROBIOTA PROFILES

Navarro-Guillén, C.[1], Huesa-Cerdán, R. [1], Hidalgo-Pérez, J.A. [1] and Perera, E. [1]

[1]*Andalusian Institute of Marine Sciences (ICMAN), Spanish National Research Council (CSIC), Cadiz, Spain*

e-mail: carmen.navarro@csic.es

Diet is a key determinant of host physiology and gut microbiota composition in fish, with nutritional compounds potentially influencing host–microbiota interactions. In the present study, we investigated the effects of dietary genistein and B-vitamins on host–microbiota interactions in gilthead seabream (*Sparus aurata*) juveniles. To address this, fish were fed three experimental diets: a control diet, a diet supplemented with genistein and an enriched diet in B-vitamins. Intestinal samples from the same individuals were subjected to RNA sequencing to characterize host transcriptional responses and 16S rRNA gene sequencing to assess shifts in gut microbial communities. More than 1,500 genes showed significant differential expression among dietary treatments. Functional enrichment analysis showed that the proteolytic process (GO:0006508) and seven molecular functions related to serine- and metallo-peptidase activity were enriched among differentially expressed genes, suggesting coordinated modulation of intracellular protein processing, intestinal extracellular matrix remodeling, and mucosal maintenance. KEGG analysis identified extracellular matrix–receptor interaction (dre04512) as the most upregulated pathway in 1C fish compared to controls, while steroid biosynthesis (dre00100) was significantly regulated across all comparisons. Interestingly, correlations between microbial taxa and host cholesterol biosynthesis pathways in the B-vitamins treatment was revealed by correlation-based integration of transcriptomic and microbiome data. Further functional integration analyses, including gene set variation analysis (GSVA), are ongoing. Likewise, in progress RRBS profiling of the same samples may uncover new host–microbiota associations in this species. These findings provide new insights into the interplay between these dietary compounds, host intestinal responses, and gut microbiota in *S. aurata*, highlighting the potential of slight dietary interventions to modulate host–microbiota interactions in aquaculture species.

**Keywords**: Intestinal transcriptomics, Gut microbiota, Multi-omics integration, Nutritional intervention, *Sparus aurata*

# TRANSCRIPTOMIC PROFILING OF FAST- AND SLOW-GROWING *SPARUS AURATA* REVEALS POTENTIAL GROWTH BIOMARKERS

Angelo, M.[1], Silva, I.A.L.[1], Barata, M.[2], Rocha, M.[1], Pousão-Ferreira, P.[1,2] and Lourenço-Marques, C.[1,2]*

[1] *S2AQUA, Olhão, Portugal;* [2] *EPPO/IPMA, Olhão, Portugal*

e-mail: morgana.angelo@s2aquacolab.pt; catia.marques@s2aquacolab.pt; *presenting author

Growth variability in gilthead seabream (*Sparus aurata*) represents a major challenge for Mediterranean aquaculture, as individuals raised under identical conditions often show large differences in growth rate. The underlying molecular causes of this variability remain poorly understood because conventional gene expression analyses target only a limited group of genes. Two trials were conducted at EPPO/IPMA facilities, where growth differences were identified at initial sampling. Then fish were sorted into slow- and fast-growing based on the average batch weight, with density maintained across all tanks. Liver and intestine (in trial 1 and 2) and muscle (only in trial 2) samples were collected from six fish per condition (fast vs slow) for differential gene expression (DGEs) analysis. Total RNA was extracted, quantified and quality verified by gel electrophoresis before sending for library construction and mRNA sequencing (Novogene Co., Ltd). To select growth biomarkers in liver and intestine, the DEGs in both trials were compared and only the ones differentially expressed in both trials were selected as hits for validation through RT-qPCR. To select growth biomarkers in muscle, the top 5 up- and down DGEs were selected as hits using data only from trial 2. Results show that fast-growing fish outperformed slow-growing ones, demonstrating better growth potential and physiological performance. The combination of both trials' biomarkers selection revealed 11 up (including *cacna1d*, *ccbe1* and *hamp*) and 4 down-regulated genes (including *efna2* and *trim21*) in liver and 1 up (*samd9*) and 2 down-regulated genes (*dhrs12* and *irgc*) in intestine, when comparing fast- with slow-growing fish. The top 5 up-regulated genes in muscle were *tmprss9, ctxn3, nlrp12, dpy30* and *znf608*, and top 5 down-regulated genes were *mfap4, dhys, mdn1, lgals3* and *gpr12*. RT-qPCR validation of some genes confirmed the differential expression between fast- and slow-growing fish, concordant with RNA-seq results. These genes play diverse yet interconnected roles in growth regulation, metabolism, immunity, and muscle function. Their differential expression between fast- and slow-growing fish suggests that they could serve as potential biomarkers for growth profiles in *Sparus aurata*. These results reveal distinct molecular signatures associated with growth capacity in gilthead seabream and that the use of RNA-seq proved to be effective for identifying genes and regulatory networks directly related to growth performance. Future work includes expanding validation efforts and conducting additional trials to further test the predictive value of these biomarkers in a real-life setting.

**Keywords**: Gilthead seabream, RNA-Seq, Gene expression, Liver, Intestine, Muscle

**Acknowledgments:** This study was funded by the project NanoPEIXE (ALG-01-0247-FEDER-070032) and with funding from Interface Mission, project cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148).

# PHYTOGENIC STRATEGIES TO ENHANCE GUT RESILIENCE IN SEABREAM

Sarropoulou, E.[1] *, Kokou F.[2], Markomanolaki H[1]., Mastoraki M.[1], Chatzifotiou S. [1], Chabrillat T. [2], Kerros S.[3] and Fagnon M. S[3]

*[1]Hellenic Center for Marine Research, P.O. Box 2214, Heraklion, Crete 71003, Greece, [2]Wageningen University, Animal Science Department, De Elst 1, Wageningen, Netherland, [3] Phytosynthese, 57 Avenue Jean Jaurès, 63200 Mozac, France.*

e-mail: sarris@hcmr.gr

Cold stress profoundly reprograms intestinal molecular networks in gilthead seabream (*Sparus aurata*), and dietary phytogenics selectively engage these responses. We used deep 3'UTR RNA-seq (672M reads, ~87% mapping) and distal-intestine 16S profiling to test Brazilian green propolis (PR), turmeric (TC), and their combination (TL) at ambient (23 °C) and winter-simulating temperature (13 °C). At ambient temperature, PR promoted pathways converting nutrients and small molecules (e.g., retinol, beta-alanine, carbon substrates) into energy, biosynthetic precursors, and signaling molecules for cellular homeostasis. TC produced a stronger effect with 722 DEGs showing broad metabolic and immune signatures. TL had minimal effects at 23 °C but triggered a cold-specific response (292 DEGs) at 13 °C, with lysosome and sphingolipid metabolism pathways being down-regulated, suggesting reduced lipid signaling/turnover and intracellular degradation/recycling. Under cold stress TC markedly activated circadian clock–linked genes, revealing a temperature–diet interaction that engages clock-related transcriptional networks. Microbiome analyses showed temperature- and diet-dependent restructuring: additive diets (especially Turmelis, less so turmeric) decreased Proteobacteria and increased Bacteroidetes and Firmicutes at 23 °C; changes at 13 °C were subtler with dominant taxa largely stable. Bacteroides and Alkalibacterium dominated across both experiments. Overall, turmeric notably amplifies intestinal circadian circuitry under low-temperature stress, potentially reprogramming temporal control of metabolism and immunity; modulating the gut clock and microbiota is a promising chrononutrition strategy to boost seabream metabolic resilience and immune competence in winter.

**Keywords**: RNA-Seq, Microbiome, Teleost, Nutrition, Cold stress, Additives

# MILD OXIDATIVE STRESS GUIDES DIETARY EPIGENETIC MODULATORS TO DIRECT DNA METHYLATION TOWARD STRESS-RESILIENCE PATHWAYS

Perera, E.[1], Navarro-Guillén, C.[1], Huesa-Cerdán, R.[1], Hidalgo-Perez, JA.[1], Martínez-Rodríguez, G[1] and Rodriguez-Casariego, JA.[2]

[1] *Andalusian Institute of Marine Sciences (ICMAN), Spanish National Research Council (CSIC), Cadiz, Spain;* [2] *Institute of Environment, Florida International University, Miami, Florida, USA.*

e-mail: erick.perera@csic.es

Although hepatic epigenetic plasticity to dietary stimuli is well established, the factors that bias methylation remodeling toward specific processes remain unknown. We hypothesized that mild oxidative stress acts as a contextual cue directing dietary epigenetic modulators toward DNA methylation reprogramming of stress-resilience pathways. To test this model, we analyzed hepatic transcriptional and DNA methylation responses to a genistein/vitamin C diet in seabream. RNA-seq identified 146 differentially expressed genes (DEGs), with upregulated genes involved in SAM metabolism, chromatin remodeling, protein catabolism, and mitochondrial activity, and downregulated genes linked to glucose and lipid metabolism, intracellular trafficking, and signaling. Weighted gene co-expression network analysis revealed modular transcriptomic responses associated with liver weight, plasma cortisol, and oxidative stress traits, suggesting coordinated regulation of metabolic, antioxidant, and signaling pathways. Reduced representation bisulfite sequencing (RRBS) identified 16,300 differentially methylated cytosines and 354 differentially methylated regions, primarily in gene bodies and intergenic regions. Moreover, Functional Epigenetic Module analysis showed promoter DNA methylation changes affecting epigenetic regulation, oxidative stress and stress adaptation. This integrative analysis suggested that methylation changes modulate functionally related gene networks, contributing to redox and metabolic adaptation. Thus, diet-induced methylation changes were not randomly distributed but preferentially occurred in genomic regions associated with cellular stress responses, suggesting selective reprogramming rather than global remodeling. We propose that mild oxidative stress serves as a contextual signal, guiding dietary epigenetic modulators to reshape DNA methylation landscapes in a pathway-specific manner. Subsequent exposure to a ROS-generating challenge revealed a faster and more efficient oxidative stress control in epigenetically remodeled livers. Our findings provide a new perspective for precision nutrition and the epigenomic reprogramming of fish traits of interest in aquaculture.

**Keywords:** DNA methylation, Epigenetic reprogramming, Gene regulatory networks, Nutritional epigenetics, Phenotypic plasticity, Redox signaling, Stress resilience

# EPIGENETIC BASIS OF THE ONSET OF SEXUAL SIZE DIMORPHISM IN TELEOSTS

Ecker-Eckhofen, G.[1], López, N.[1], Beato, S.[1], Allal, F.[2], Vandeputte, M.[2] and Piferrer, F.[1]

[1]*Institute of Marine Science (ICM), Barcelona, Spain,* [2]*MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, INRAE, Palavas-les-Flots, France*

e-mail: eckhofen@icm.csic.es

Numerous economically important farmed fish, such as the European seabass (*Dicentrarchus labrax*), Nile tilapia (*Oreochromis niloticus*) and Chinese tongue sole (*Cynoglossus semilaevis*), exhibit sexual size dimorphism (SSD) whereby one sex grows larger than the other. This trait plays a significant role in aquaculture, where selection for the faster-growing sex is common practice. While DNA methylation (DNAme) is recognized to be a key driver of phenotypic plasticity, identifying the epigenetic signatures of SSD remains difficult. The primary challenge lies in disentangling DNAme patterns that are sex-related from growth-related differences, as both seem to be deeply interconnected. This is especially true for species that lack sex chromosomes and rely on polygenic sex determination, such as the European seabass. To address the challenging sex-growth entanglement, we employed a strategy to isolate both variables. Similar-sized seabass were sampled across five time points covering the onset and divergence of SSD (102–368 dpf). We then utilized Enzymatic Methyl-seq to profile whole-genome methylomes of liver tissues. By obtaining both same-sized and differently-sized individuals of both sexes at each time point, this design allows for dissociating growth-related from sex-related DNAme. Here, we present what is known about SSD-associated genomic signatures as well as preliminary findings of this study. Ultimately, understanding these epigenetic drivers would allow for more precise sex control and growth selection strategies in commercial production of fish with sexual size divergence.

**Keywords**: EM-seq, Methylome, European Seabass, Sexual Size Dimorphism

# SINGLE-NUCLEUS TRANSCRIPTOMICS OF ATLANTIC SALMON MUSCLE CAPTURES MYOGENIC CELL STATES AND POST-DUPLICATION DIVERGENCE

Sobhiafshar, U. [1], Lan, L.[1], Taylor, R.S.[1], Furniss, J.J.[1], Sun, J.[1], Macqueen, D.J.[1] and Vernimmen, D.[1]

[1]*The Roslin Institute, Edinburgh, Scotland*

e-mail: usobhiaf@ed.ac.uk , douglas.vernimmen@roslin.ed.ac.uk

Salmonids are among the most valued teleost species in global aquaculture, but also an emerging system to study genome evolution. Like other teleosts, muscle is the most abundant tissue in salmonids. Myofibers are formed through myogenesis, a conserved and well-orchestrated developmental process in vertebrates where myogenic progenitor cells (MPCs) proliferate and terminally differentiate to myotubes and then myofibers. Unlike mammals, salmonids exhibit indeterminate growth through sustained muscle fiber recruitment (hyperplasia) into adulthood. During vertebrate evolution, whole genome duplication (WGD) events have reshaped vertebrate gene networks and cell developmental programs. Following a teleost-specific WGD (Ts3R) event, salmonids underwent another round of WGD (Ss4R) about 100 million years ago, which created a repertoire of paralogs with subsequent potential to evolve new functions. Here, we report a single-nucleus RNA-seq study of epaxial myotomal muscle in Atlantic salmon. We have resolved eight distinct differentiation states from quiescent MPCs through to terminal myofiber formation. We provide evidence for expression divergence among paralogs of master myogenic regulators, capturing evolutionary changes in the transcriptional regulation of myogenesis following the 4R WGD. Additionally, we demonstrate evidence for distinct gene co-expression networks operating during hyperplasia and hypertrophy stages of myogenesis. Our study provides an initial foundation of cellular heterogeneity during myogenesis in salmonid fishes at the single-cell level, particularly in the context of evolutionary changes following the Ss4R WGD.

**Keywords**: Single-nucleus, snRNA-Seq, myogenesis, whole genome duplication, paralogue genes, Atlantic Salmon

## Session 5 — Microbial genomics

## **Chairs:** Kiron Viswanath, Dana Silva, Sandra López

### *FLAVOBACTERIUM PSYCHROPHILUM* GENOMICS TO UNRAVEL EVOLUTION, VIRULENCE AND HOST ADAPTATION OF A MAJOR FISH PATHOGEN

Duchaud, E.[1], Nicolas, P.[2] and Rochat, T.[1]

[1]*Université Paris-Saclay, INRAE, UVSQ, VIM, Jouy-en-Josas, France;* [2]*Université Paris-Saclay, INRAE, Malage, Jouy-en-Josas, France*

e-mail: eric.duchaud@inrae.fr

*Flavobacterium psychrophilum* is the etiological agent of rainbow trout fry syndrome and bacterial cold-water disease in salmonid fish reared in freshwater. *F. psychrophilum* is currently one of the main bacterial pathogens hampering the productivity of salmonid farming worldwide. Our team has a long-lasting "love story" with bacteria of this family ranging from classification guidelines in the late 90 (1), the very first complete genome sequence of a bacterial fish pathogen in 2007 (2), to transcriptomics (3) and phenotypic characterization of relevant bacterial isolates. Over the years, we developed molecular typing tools and conducted comparative genomic analysis. We revealed that *F. psychrophilum* is a bacterial species harbouring a limited genomic diversity in terms of both nucleotide diversity and gene repertoire and provided evidences that homologous recombination is the key driver of genome evolution. We also disclosed that bacterial isolates cluster in clonal complexes according to their host-fish species suggesting a panmictic population structure with the expansion of highly virulent epidemic clones in rainbow trout farms (4).

Combining genome-wide association studies, mutagenesis and phenotypic screens we identified relevant molecular traits explaining previously unresolved phenotypic characteristics such as serological diversity, proteolytic capabilities and nutrient acquisition systems. Last developments will be presented allowing large scale epidemiological studies and opening new avenues for a better control of this important fish pathogen.

**Keywords**: Flavobacterium, virulence, genomics, salmonids

***Reference***

(1) Bernardet JF et al. 2002. Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. Int J Syst Evol Microbiol. 52:1049-1070

(2) Duchaud E et al. 2007. Complete genome sequence of the fish pathogen Flavobacterium psychrophilum. Nat Biotechnol. 25:763-9

(3) Guérin C et al. 2021. Transcriptome architecture and regulation at environmental transitions in flavobacteria: the case of an important fish pathogen. ISME Commun. 1:33

(4) Duchaud E et al. 2018. Genomic Diversity and Evolution of the Fish Pathogen Flavobacterium psychrophilum. Front Microbiol. 9:13

# IDENTIFICATION OF AN ANTIBIOTIC RESISTANCE CLUSTER IN *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA* ASSOCIATED WITH NEW PLASMIDS AND A-LAYER EXPRESSION

Saidan, L.[1], Moreau, E.[1] and Oberlé, K.[1]

[1]*Oniris, INRAE, BIOEPAR, 44300 Nantes, France*

e-mail : line.saidan@inrae.fr

*Aeromonas salmonicida* subsp. *salmonicida* (ASS), the causative agent of furunculosis in rainbow trout (*Oncorhynchus mykiss*), poses a major risk in aquaculture. Treatment relies on a limited number of antibiotics and the emergence of resistance is gradually compromising therapeutic efficacy. This resistance may result from the presence of resistance genes carried by mobile genetic elements such as plasmids or transposons, but also from the expression of A-layer, a major virulence factor in ASS[1]. In this study, we analyzed the phenotypic and genomic determinants of antibiotic resistance in a collection of 45 ASS isolates collected in France between 2013 and 2021. Antibiotic resistance was previously assessed by determining minimum inhibitory concentrations (MICs)[2]. The expression of the A-layer in the 45 isolates was determined by culturing them on agar supplemented with Coomassie Blue. Regarding the isolates that highlight two subpopulations which express the A-layer differentially, the inhibition diameters for β-lactams were measured by agar diffusion according to CLSI VET04 for both subpopulations. Genomes were sequenced using Oxford Nanopore technology and then assembled with Autocycler. Taxonomic identification was confirmed by Average Nucleotide Identity (ANI) and digital DNA-DNA hybridization (dDDH) values. Resistance genes were searched for using BLASTp against a customized database, and plasmids were characterized using BLASTn, Proksee, PlasFlow, and MOB-suite. All strains were confirmed as belonging to ASS, based on ANI and dDDH values greater than 99% and 90%, respectively. Several resistance genes were detected heterogeneously, including *floR* and its variant *floR_2 (ID = 95.30% and cov = 100%)*, *tetA*, *tetE*, *tetC*, *tetR*, and *sul1*. Some of these resistance genes were found in known plasmids, such as pAsa5 and pAsa4. Three new plasmids have been identified, pAsa13 and its variants, carrying *sul1*, *floR*, *tetR*, and *tetA*, associated with IS91 insertion sequences and Tn3-type transposases, pAsa14, carrying *floR_2*, and pAsa15, carrying *tetA*, *tetR* and *sul1*, associated with Tn3-type transposases. Two of them, pAsa14 and pAsa15, were predicted to be conjugative by MOB-suite. Mixed data factor analysis, comparing MICs and the presence of resistance genes, divided the isolates into three clusters. Cluster 3 had the highest MICs, carried several resistance genes (*tetA*, *tetR*, and *floR)* located on new plasmids and was mainly composed of A-layer- isolates. Additionally, nine isolates showing two subpopulations with differential expression of A-layer from one clone demonstrated greater ampicillin and cephalexin inhibition diameters in the A-layer$^-$ subpopulation than in the A-layer+. (Wilcoxon test; $p = 0.022$ and $p = 0.042$, respectively). The phenomenon of antibiotic resistance in *Aeromonas salmonicida* subsp. *salmonicida* appears to be due to resistance genes located on potentially conjugative plasmids, which highlights the importance of surveillance in a One Health approach. Further investigations into resistance associated with the A-layer, which is differentially expressed from a clone, should be conducted.

**Keywords**: Antibiotic resistance, Plasmids, A-layer, *Aeromonas salmonicida* subsp. *salmonicida*, Rainbow trout

***Reference***

1. Henry, M. A. & Secombes, C. J. (2000). Fish Shellfish Immunol 10:637–642.

2. A. Rostang et al. (2025). Front Microbiol 16:1532748.

# PHOTOPERIOD-DRIVEN SEX-SPECIFIC SHIFTS IN BACTERIAL AND FUNGAL GUT COMMUNITIES IN ATLANTIC COD (*GADUS MORHUA*)

Silva, D.M.[1], Colonna, L.[2], Konstantinidis, I.[2], Siriyappagouder, P.[2], Bisa, S.[2], Viswanath, K.[2], and Fernandes, J.M.O.[1]

*[1]Renewable Marine Resources, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain, [2]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway*

**e-mail:** danasilva@icm.csic.es

The gut microbiota is significantly modulated by environmental signals such as photoperiod. Acting as a 'Zeitgeber', this powerful cue influences the internal circadian rhythm of hosts, which in turn affects gut microbiota rhythmicity and host physiology. The rhythmicity of gut microbiota has also been reported to be influenced by sex. Despite its importance, relatively few studies have investigated the extent to which photoperiod induces sex-specific shifts in the gut microbiota. The present study aimed to investigate such shifts in the gut microbiota of Atlantic cod (*Gadus morhua*) exposed to different photoperiods. Juvenile Atlantic cod (both males and females) were assigned to two experimental groups: males and females were exposed to either continuous light (24L:0D; CL males and CL females) or a photoperiod of 12L:12D (AL; AL males and AL females). After the experimental period of six months, gut samples (15 males and 15 females per condition) were collected for microbial characterization. Bacterial and fungal communities were characterized via high-throughput sequencing of the 16S rRNA gene and ITS2 region, respectively. Alpha diversity analysis (Observed, Chao1, and Faith PD) revealed a significant interaction between photoperiod and sex ($p < 0.01$). Although no significant differences were observed between males and females under ambient light (AL_F and AL_M), the application of continuous light induced a sex dependent response. Beta diversity analysis confirmed significant changes in the community composition (PERMANOVA, $p < 0.05$), and the CL females showed the most marked microbial divergence compared to CL males and AL females. Relative abundance analysis of microbial communities revealed that females exposed to CL were characterized by a massive enrichment of *Brevinema* (50.6%), while males exposed to CL showed a predominance of *Vibrio* (42.6%), a genus often associated with opportunistic pathogens. The fungal communities remained remarkably stable, with no significant differences in alpha or beta diversity and a core microbiota dominated by *Malassezia* and *Ustilago* (100% prevalence). Nevertheless, *Ustilago* showed plasticity, with decreasing relative abundance in males exposed to CL (11.3%). In summary, the mycobiota exhibited greater stability, with subtle changes in males exposed to continuous light, while distinct sex-specific bacterial signatures were evident during continuous light exposure.

***Keywords:*** Gut microbiota, mycobiota, bacteria, sexual dimorphism, light

***Acknowledgments:*** This study received funding from the Research Council of Norway under the Researcher Project for Scientific Renewal (EPICOD, project No. 336112), the Spanish Ministry of Science and Innovation grant PID2023-146286OB-I00 "Holosex" and FPI contract PRE2022-101795 associated to grant CEX2019-000928-S-20-5 funded by AEI 10.13039/501100011033 (Spain).

# THERMAL AND DEVELOPMENTAL DRIVERS OF MICROBIAL COMMUNITY DYNAMICS IN GILL, INTESTINE AND SKIN OF GILTHEAD SEA BREAM AND EUROPEAN SEABASS DURING EARLY ONTOGENY

Abdollahpour, H.[1], Papandroulakis, N.[2], Moutou, K.[3], Tsipourlianos, A.[3] and Power, D.M.[1]

*[1]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Faro, Portugal; [2]HCMR, Crete, Greece; [3]University of Thessaly, Biopolis,Larissa, Greece*

e-mail: habdollahpour@ualg.pt; dpower@ualg.pt

The microbiome is critical for fish health; however, the mechanisms by which it is established in early life under elevated temperature stress remain largely unresolved for aquaculture. This study investigated the individual and combined effects of the thermal regime from egg to mouth open stages on microbiome assembly in two stages of the commercially important teleosts, gilthead sea bream (*Sparus aurata,* SA) and European seabass (*Dicentrarchus labrax,* DL). Fertilized eggs were incubated under species-specific thermal regimes (sea bream: 17, 20, 23°C; seabass: 14, 17, 20°C) until larval mouth opening after which the temperature was standardized to 20°C for sea bream and 17°C for sea bass and subsequently sampled at the end of larval rearing (ER) and mid-metamorphosis (MM). The gill, intestine, and skin were microdissected out, genomic DNA extracted and 16S rRNA gene amplicon sequencing performed. The results revealed species-, tissue-, and stage-dependent effects of temperature. In sea bream, temperature was a primary driver of beta diversity in the gill and skin at ER, but this effect diminished in MM. In DL, temperature significantly structured intestinal and gill communities at the MM stage. Metamorphosis was the dominant factor driving significant microbial restructuring in all tissues of SA and DL. Alpha diversity shifts were tissue-dependent, and SA skin showed a marked loss of richness, while DL skin showed a significant increase. Taxonomically, both species exhibited a developmental shift away from early *Vibrio* dominance, with the emergence of tissue-specific genera such as *Polaribacter* in SA gills and unclassified *Candidatus Campbellbacteria* in DL skin. The results uncovered a plastic microbiome influenced by the experienced early thermal regimes and was highly dependent on host species, tissue niche, and developmental stage. This study provides a critical baseline for understanding how environmental change modulates host–microbe interactions during larval development, with implications for aquaculture management and species resilience in a warming climate.

**Keywords:** fish microbiome, thermal adaptation, metamorphosis, *Sparus aurata*, *Dicentrarchus labrax*, aquaculture

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

# EFFECTS OF A DIETARY BACTERIOPHAGE TARGETING *FLAVOBACTERIUM PSYCHROPHILUM* ON THE GUT MICROBIOME OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Overland, B.[1], Shankregowda, A.M.[1], James, L.[1], Uren Webster, T.M.[1], Ruffo, V.[2], Middelboe, M.[2], Hitchings, M.[3], Garcia de Leaniz, C.[4] and Consuegra, S.[1,5]

*[1]Department of Biosciences, Centre for Sustainable Aquatic Research, Swansea University, Swansea, UK, SA2 8PP, [2]Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark, [3]Institute of Life Science, Swansea University, Swansea, Wales, SA2 8PP, UK, [4]Centro de Investigaciones Marinas, Universidade de Vigo, Vigo, Spain, [5]Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain.*

email: b.j.overland@swansea.ac.uk

The global trade of salmonids is increasing rapidly, causing a shift towards more intensive aquaculture systems that can increase the prevalence and intensity of disease outbreaks. *Flavobacterium psychrophilum* represents one of the most important pathogens in global aquaculture, causing both bacterial cold-water disease and rainbow trout (*Oncorhynchus mykiss*) fry syndrome. Egg disinfection and antibiotic treatment are widely used to prevent and control diseases, however exposure to antibiotics can lead to the development of antibiotic resistance, and both methods can disrupt the microbiome, a critical regulator of fish health and immune function. Bacteriophages (phages) are natural, antibacterial agents which can be highly specific. As the rate of antibiotic resistance increases globally, the application of targeted phages is becoming more common, and phages targeting major pathogens, including *Aeromonas salmonicida*, *Yersinia ruckeri* and *Flavobacterium psychrophylum*, have been used with reported success. However, despite their specificity, the effects of phage therapy on the composition and diversity of the gut microbiome are not yet known.

Through an early life disinfection, to simulate common aquaculture practices, followed by the targeted application of phages specific to *F. psychrophilum* (FPV-4), we examined the corresponding changes to the rainbow trout gut microbiome. Phages were supplemented to the diet for 80 days. Gut microbiome samples were collected at three developmental stages: 50 days, 80 days and 120 days (30 days after phage feeding ended), and microbial community composition was analysed using 16S rRNA sequencing. Initial results indicate that the gut microbiome naturally changes over time, but phage treatment alters the rate of this change, producing clear but time-dependent shifts in gut microbiota community structure and alpha diversity.

**Key words**: *Oncorhynchus mykiss*, gut microbiome, phage treatment, *Flavobacterium psychrophylum*.

# PRIMING THE FISH MICROBIOME IN EARLY LIFE TO IMPROVE STRESSOR RESILIENCE

Uren Webster, T.M.[1], Overland, B.[1], Shankregowda, A.M.[1], Gwynedd, E.[1], Laing, L.[2], Hitchings, M.[3], Garcia de Leaniz, C.[1,4], Santos, E.M.[2] and Consuegra, S.[1,5]

*[1]Biosciences, Faculty of Science & Engineering, Swansea University, Swansea, UK, [2]Biosciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, UK, [3]Institute of Life Sciences, Swansea University, Swansea, UK, [4]Centro de Investigaciones Marinas, Universidade de Vigo, Vigo, Spain, [5]Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain,*

e-mail: t.m.urenwebster@swansea.ac.uk

The microbiome plays a critical role in host health, influencing digestion and nutrient uptake, metabolism, immune function, and pathogen defence. Promoting the abundance of beneficial bacteria within the microbiome, in order to enhance health and disease resistance in aquaculture species, is therefore an active and promising area of research. However, successfully introducing new probiotic bacteria into an established microbial community can be challenging, as microbiome structure and function are governed by a complex interplay of environmental, microbial, and host factors.

A major focus of our recent work has been the microbiome during early developmental stages. The initial period of microbial colonisation and proliferation on host mucosal surfaces represents a window of heightened microbiome sensitivity, during which early colonisers gain a competitive advantage through priority effects. Across several species, including Atlantic salmon and rainbow trout, we have shown that stressors applied during early life can have persistent effects on microbiome diversity and composition, including increases in opportunistic pathogens. In contrast, these same sensitive early life stages also present a promising opportunity to condition or prime the microbiome with probiotic bacteria.

Priming the holobiont during early development is another promising approach. Microbiota have a substantial capacity to develop tolerance to environmental stressors, and this can extend host adaptive plasticity by providing specific metabolic functions and/or reducing the likelihood of adverse physiological effects associated with microbiome dysbiosis. We have recently demonstrated that developmental priming can improve copper tolerance in the stickleback microbiome, which likely contributes to enhanced copper tolerance in the host as well.

**Keywords:** Microbiome, Stress, Developmental plasticity, Probiotic, Priming

# DAY 3 — Wednesday, 6 May 2026

## Session 6 — Integrative omics

**Chairs:** Paulino Martinez, Arun Shankregowda

# GENOME ARCHITECTURE SHAPES EPIGENETIC AND TRANSCRIPTIONAL DIVERGENCE IN EUROPEAN SEABASS POPULATIONS

Sánchez-Baizán, N.[1], Vandeputte, M.[1] and Allal, F.[1]

[1]*MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, INRAE, Palavas-les-Flots, France*

e-mail: Francois.Allal@ifremer.fr

Beyond genetic variation, epigenetic mechanisms such as DNA methylation can modulate gene expression and integrate both genetic background and environmental history contributing to phenotypic differences. However, how epigenetic divergence relates to genome architecture and population structure remains poorly understood. Here, we investigated the interplay between genome architecture, epigenetic divergence, and phenotypic variation in European seabass (*Dicentrarchus labrax)*. We used three populations—Atlantic (AT), Eastern Mediterranean (EM), and an admixed Western Mediterranean (WM)—reared under a common-garden experimental design representative of the EM thermal regime. Using transcriptomic and whole-genome DNA methylation data from liver and muscle, we quantified tissue-specific epigenetic and transcriptomic divergence.

Both molecular layers captured population divergence, with the highest observed in the liver. Functional analyses revealed differences in metabolic regulation, cellular homeostasis, and immune-related pathways, while co-expression network analyses showed that core growth-related networks were largely shared across populations. We examined whether epigenetic variation was constrained by genome architecture through permutation-based analysis. Whole genome DNA methylation was strongly shaped by the recombination landscape and admixture regions, revealing some population-specific differences. In the admixed population, DNA methylation was strongly associated with local ancestry, indicating a tight link between epigenetic variation and genetic background. Overall, our results showed that genome architecture shaped epigenetic variation in European seabass, providing new insights into how genetic and epigenetic variation jointly contribute to population-level phenotypic divergence, with implications for understanding robustness and adaptive potential in aquaculture species.

**Keywords**: DNA methylation, recombination rate, admixture.

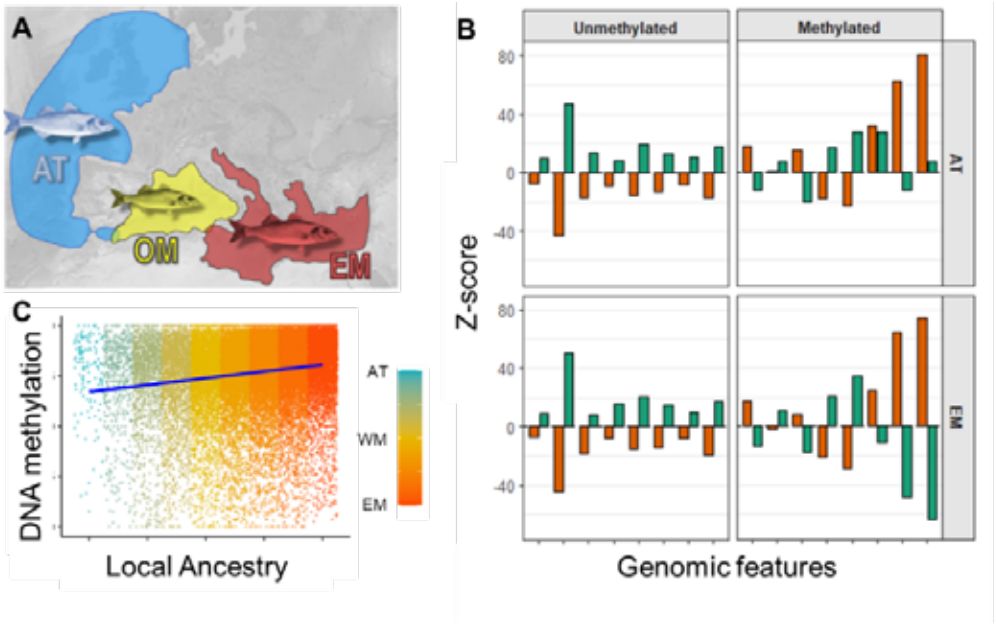



***Figure 2*** *A) Distribution of the three populations of European seabass. B) Enrichment of methylation levels overlapping with high (orange) and low (green) recombination rate in the AT and EM populations. C) Local ancestry predicting DNA methylation in the admixed population.*

# CELL-TYPE-SPECIFIC ENDOCRINE SIGNALS OF THE PITUITARY GLAND IN RESPONSE TO PHOTOPERIOD MANIPULATION

Konstantinidis I. [1], Colonna, L. [1], Daniels, R.R. [2], Robledo, D. [3] and Fernandes, J.M.O. [4]

*[1]Nord University (FBA), Bodø, Norway; [2]University of Stirling, Stirling, Scotland, [3] University of Santiago de Compostela, Santiago de Compostela, Spain, [4]Institute of Marine Science (ICM), Barcelona, Spain.*

e-mail: ioannis.konstantinidis@nord.no

The onset of puberty in farmed fish species brings about a set of phenotypic and behavioural changes that can negatively impact productivity and the environment. Although the onset of puberty can be controlled to some extent via photoperiod and temperature manipulation, their application in industrial-scale sea cages remains limited. As puberty is largely influenced by external stimuli, we hypothesize that transcriptional and epigenetic signals in key tissues, such as the pituitary gland, can shed light on the fundamental biological networks that regulate puberty. To address this, Nile tilapia (*Oreochromis niloticus*) were exposed to continuous or ambient light for four months and their morphometric characteristics were recorded.

To capture in-depth the molecular mechanisms that underlie puberty, we employed state-of-the-art single-cell multiome profiling (10x Genomics), simultaneously assessing gene expression (RNA-seq) and chromatin accessibility (ATAC-seq) within the same cells. This approach enabled the identification of major pituitary cell types with distinct gene expression and chromatin accessibility profiles. Through differential gene expression and differential chromatin accessibility analyses, combined with transcription factor motif enrichment and their correlations, we identified cell-type-specific regulatory programs and candidate genes associated with endocrine signaling and reproductive maturation. Importantly, several genes and regulatory regions showing coordinated changes in chromatin accessibility and transcription were identified within specific pituitary cell populations, suggesting epigenetic mechanisms that may influence reproductive axis activation. These results provide a valuable baseline for interspecies comparisons, and optimization of single-cell analysis and cell-type annotation in commercially important fish species, such as Atlantic cod (*Gadus morhua*) and European seabass (*Dicentrarchus labrax*).

**Keywords**: Single-cell, RNA-Seq, ATAC-Seq, Teleost, Puberty

**Acknowledgements:** This study has received funding from the European Research Council (project nos. 683210 and 101081935), Nord University Research & Development Division (AFU; Ref:22/00230-15/), the Research Council of Norway (project No. 336112), the Spanish Ministry of Science and Innovation grant PID2023-146286OB-I00 “Holosex” and grant CEX2019-000928-S funded by AEI 10.13039/501100011033 (Spain).

# PHOTOPERIOD EFFECT ON ATLANTIC COD GONADS AND PITUITARY REVEALS MODULATION OF GENE EXPRESSION AND METHYLATION

Colonna, L.[1*], Konstantinidis, I.[1], Silva, D.M.[2], Puvanendran, V.[3], Siriyappagouder, P.[1], Hansen, Ø.J.[3] and Fernandes, J.M.O.[2]

*[1]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway, [2]Renewable Marine Resources, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain, [3]NOFIMA AS, Tromsø, Norway*

e-mail: lorenzo.colonna@nord.no

Light serves as fundamental regulator of biological cycles across taxa, not only modulating behavioural patterns but also physiological processes and metabolic pathways. Within teleost aquaculture, the influence of photoperiod on growth and development is well-known. Atlantic cod represents a historically valuable species for commercial farming; however, despite the recent advances in our understanding of its biology, early sexual maturation still remains a primary bottleneck to large scale production. Past research confirms that manipulation of photoperiod regimes can mitigate the precocious onset of puberty in cod, and current evidence suggests that this external stimulus could trigger transcriptional and epigenetic shifts within the hypothalamic-pituitary-gonadal axis. Consequently, the present study aims to identify the molecular drivers of this phenomenon through a multi-omic approach by integrating gene expression analysis with DNA methylation profiling. Analyses were conducted on the pituitary and gonads—tissues critical to the maturation-growth trade-off—using bulk RNA-sequencing and Enzymatic Methyl-sequencing. Experimental groups, comprising both male and female Atlantic cod were maintained under either natural light cycle or a continuous light regime. Both analytical approaches revealed significant molecular divergence between the two groups across both tissues, suggesting that external light stimuli may influence several regulatory pathways within the pituitary and gonadal tissues, while also identifying a subset of overlapping genes common to both methodologies.

**Keywords:** genomics, methylation, pituitary, gonads, aquaculture.

**Acknowledgements:** This study has received funding by Nord University and the Research Council of Norway (RCN) under the Researcher Project for Scientific Renewal (project No. 336112).

# INTEGRATIVE OMICS ANALYSES REVEAL GENOMIC DIFFERENTIATION AND DIVERGENT GENE EXPRESSION ACROSS BROWN TROUT LINEAGES

Vera, M. [1], Aramburu, O. [1], Pita, A. [2], Blanco, A. [1], Morata, J. [3], Bekkevold D [4], Heras, S. [2], Abras, A. [2], Roldán, M.I. [2], Díez-del-Molino D. [5] and García-Marín, J.L. [2]

*[1]University of Santiago de Compostela (USC), Lugo, Spain; [2]University of Girona (UdG), Girona, Spain; [3]Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain; [4]Danish Technical University (DTU), Silkeborg, Denmark; [5]Stockholm University (SU), Stockholm, Sweden.*

e-mail: manuel.vera@usc.es

Brown trout (*Salmo trutta*) exhibits a complex phylogeographic structure shaped by historical isolation, local adaptation, and extensive stocking with Central European hatchery strains, which has promoted variable levels of genetic introgression into native populations. In this study, we combined whole-genome sequencing with RNA-seq analyses to examine genomic differentiation and gene expression divergence between native Iberian lineages and hatchery-derived stocks. Genomic analyses revealed strong lineage-specific structure, finding some slight differences between mitochondrial and nuclear analyses in populations showing signatures of hybridization. Transcriptomic analyses performed on captive-reared descendants of native fish and hatchery stocks maintained under similar rearing conditions showed pronounced differences in gene expression profiles at different developmental stages (i.e., larvae, 0+ and 1+). These differences persisted despite identical environmental conditions, suggesting heritable gene expression divergence among lineages. Differentially expressed genes were primarily associated with catabolism, immune system, responses for stimuli and stress, cell communication, metabolism and energy production, pointing to contrasting functional strategies linked to their evolutionary backgrounds and to long-term exposure to either natural or hatchery environments. These results provide robust integrative omics evidence for substantial genomic and functional divergence among brown trout lineages and demonstrate that native lineages retain distinct, biologically meaningful adaptive potential. From a management and aquaculture perspective, our findings highlight that reinforcement programs and the development of broodstocks should prioritize native populations, as they harbor lineage-specific traits relevant for resilience and long-term population sustainability. This work underscores the importance of incorporating genomic and transcriptomic information into evidence-based strategies for conserving and managing *Salmo trutta* stocks.

**Keywords**: *Salmo trutta*, Genomics, Transcriptomics, Introgression, Management

# METHODOLOGICAL INSIGHTS FOR STUDYING LNCRNAS IN FISH: BENCHMARKING TRANSCRIPTOME DEPTH AND EXTRACELLULAR VESICLE RNA PROFILING

Bonnefous, L.[1], Espuela-Ortiz, A.[1], Sosa-Garcia, A.[1], Gonzalez-Montelongo, R.[2], Martín, M.V.[3], Jeréz, S.[3], Antón-González, E.[1], and Rodriguez-Barreto, D. [1,3]

*[1]Department of Animal Biology, Soil Science and Geology, Universidad de La Laguna. Astrofísico Francisco Sánchez s/n Avenue, 38206 San Cristóbal de La Laguna, Tenerife, Spain; [2]Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain; [3]Canary Islands Oceanographic Center (COC), IEO-CSIC. Farola del Mar 22, Dársena Pesquera. 38180, Santa Cruz de Tenerife, Spain.*

e-mail: deirod@ull.edu.es

Long non-coding RNAs (lncRNAs) play critical regulatory roles in fish growth, metabolism, and stress response, yet their detection and characterization remain challenging due to low expression levels and tissue-specificity (Zhou et al., 2023). In this contribution, we present methodological insights from two complementary studies aimed at optimizing lncRNA detection in teleosts, essential to ensure reliable and biologically meaningful conclusions.

First, we benchmarked the effect of sequencing depth on mRNA and lncRNA detection in *Oreochromis niloticus* (Nile tilapia) liver using Oxford Nanopore Technologies longread transcriptome sequencing. Subsampling analyses across multiple depths (10×–Full coverage [866×]) revealed that mRNA detection saturates at moderate depths (~50–100×), whereas comprehensive lncRNA discovery requires higher coverage (>100×). A protocol of Capture Long-Seq (CLS) targeting annotated lncRNAs is currently being implemented, and we expect it to markedly improve lncRNA sensitivity, allowing higher multiplexing and cost-efficiency while preserving genelevel differential expression analyses.

Second, we evaluated extracellular vesicle (EV)-derived RNA (EV-RNA) from *Sparus aurata* (gilthead sea bream) plasma to explore pre-analytical effects (pre-processing freezing and input volume) on RNA yield and integrity, long RNA composition, and lncRNAs detectability. Freezing preserved overall transcript identity but increased the proportion of shorter, incomplete isoforms. Long-read sequencing revealed EV-RNA is dominated by ribosomal and metabolic mRNAs. A total of 302 distinct lncRNAs were identified, two of which accounted for nearly 90% of lncRNA reads. Predicted interactions between abundant lncRNAs, and EV mRNAs and their encoded ribonucleoproteins suggest coordinated packaging of RNA–protein modules.

Together, these studies provide practical guidance for designing sequencing experiments targeting lncRNAs in fish, highlighting the interplay between sequencing depth, library preparation strategy, biological replication, and pre-analytical considerations. Our findings inform cost-effective approaches for liver and plasma EV lncRNA studies, supporting future functional and biomarker research in aquaculture species.

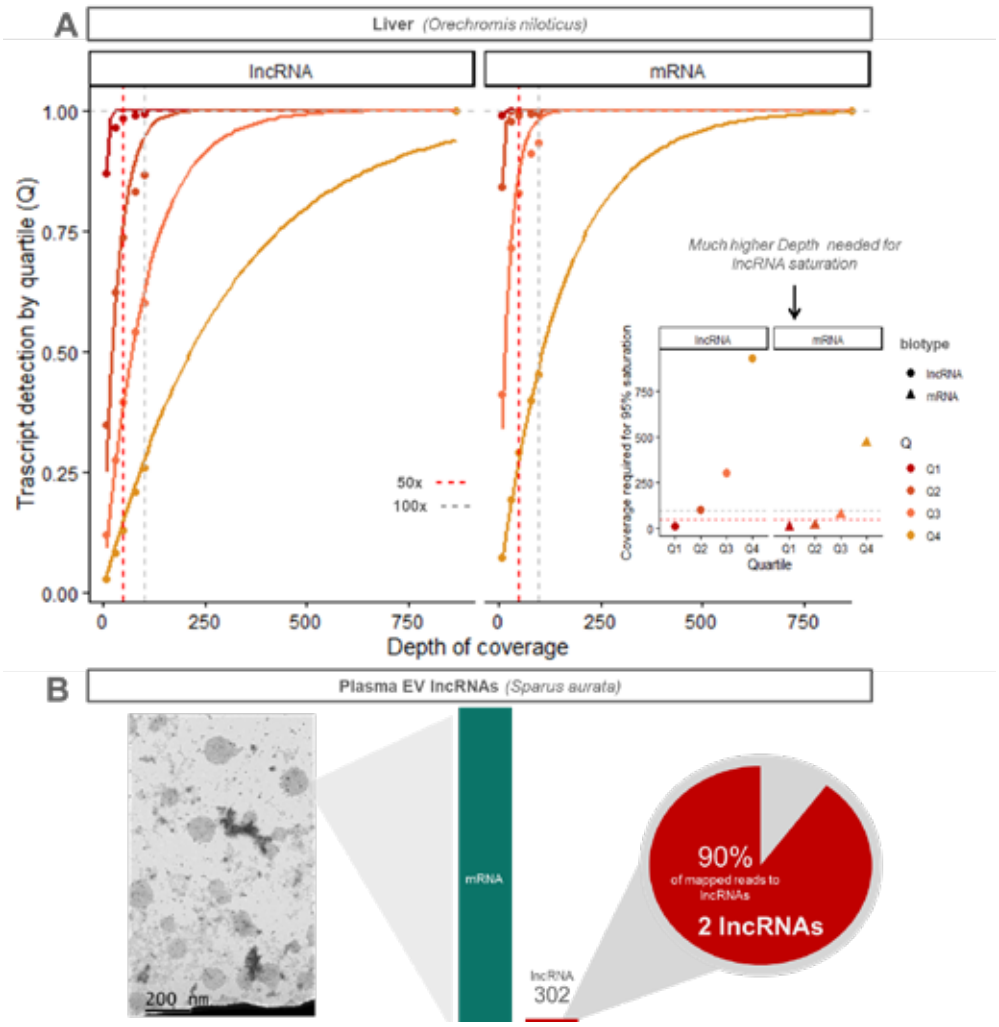



***Figure 1. (A)*** *Sequencing depth effects on lncRNA and mRNA detection in fish liver (Oreochromis niloticus);* **(B)** *lncRNA detection in plasma EVs (Sparus aurata).*

## *Reference*

Zhou Z, Leng C, Wang Z, Long L, Lv Y, Gao Z, Wang Y, Wang S, Li P. (2023) Front Immunol. 21;14:1065357. doi: 10.3389/fimmu.2023.1065357.

# SHELL-ULAR BIOLOGY: SINGLE NUCLEI-RESOLVED GENE EXPRESSION CHANGES IN THE EPITHELIAL TISSUE OF MOULTING *LITOPENAEUS VANNAMEI*

Clark, T.C.[1], Campli, G.[2,3], Dongwenjun, Z.[1], Furniss, J.[1], Waterhouse, R.[2,3] and Wade, N.[1]

*[1]The Roslin Institute, Edinburgh, Scotland; [2]University of Lausanne, Lausanne, Switzerland; [3]Swiss Institute of Bioinformatics, Lausanne, Switzerland*

e-mail: tclark3@ed.ac.uk

The exoskeleton is a defining feature of all arthropods, encasing the organism's body in a hardened outer shell composed primarily of cuticular proteins and chitin. To grow, arthropods undergo periodic shedding and replacement of the exoskeleton with a larger one in a process known as moulting (ecdysis). This process proceeds through a series of stages, from preparatory pre-moult phases to ecdysis itself, followed by post-moult maturation of the new exoskeleton. Moulting integrates physiological, environmental, and energetic cues through tightly regulated hormonal and molecular cascades that coordinate reconstruction and regeneration of the entire exoskeleton. Modern bulk transcriptomic and proteomic approaches have identified many of the hormonal regulatory mechanisms underlying this process. However, these mechanisms have not yet been resolved at the cellular level, and the cell types and molecular processes driving these phenotypic changes remain poorly understood. In the present study, we performed an integrated analysis combining histology, single-nucleus RNA sequencing (snRNA-seq), and in-situ hybridisation chain reaction (HCR) to identify epithelial cell types and characterise their changes across key stages of the *Litopenaeus vannamei* moult cycle. We identified 14 distinct cell clusters corresponding to stromal, epithelial, muscle, and two specialised clusters of cuticle- and chitin-depositing cell types. The most pronounced gene expression changes occurred immediately following moulting (post-moult) and during the late premoult phase (D3), with the cuticle-associated cell types contributing the majority of differentially expressed genes. Combined histological and in situ analyses revealed that these cuticle-depositing cell types form a dense, single-cell layer directly beneath the exoskeleton. Further analysis indicated a stage-dependent transcriptomic shift in these cells across the moult cycle, with cells alternating between expression programmes associated with cuticular protein deposition and chitin synthesis. These findings suggest that these specialised epithelial cuticle cells directly drive exoskeleton formation during the moulting process.

**Keywords**: Single-nuclei RNA-Seq, Whiteleg Shrimp, Moulting

# SKIN MUCUS AND TISSUE MULTIOMICS COMPARISON OF TRANSPORT-INDUCED ACUTE STRESS RESPONSES IN ATLANTIC SALMON, EUROPEAN SEABASS AND RAINBOW TROUT

Buha, T. [1,2,3*], Reis, B. [1], Themudo, G. [1], Cunha, F. [1], Pimentel , C. [1,2,4], Simó-Mirabet, P. [6], Martos-Sitcha, J.A. [6], Rema, P. [5], Rocha, R. [4], Costas, B. [1,2], Raposo de Magalhães, C. [3,4] and Gonçalves, A.T. [3,4]

*[1]Interdisciplinary Centre of Marine and Environmental Research of the University of Porto (CIIMAR), Matosinhos, Portugal; [2]Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto (UP), Porto, Portugal; [3]SPAROS Lda., Olhão, Portugal; [4]Riasearch Lda., Murtosa, Portugal; [5]University of Trás-os-Montes e Alto Douro, Vila Real, Portugal; [6]Instituto Universitario de Investigación Marina (INMAR), CEIMAR-Universidad de Cádiz, Cádiz, Spain.*

e-mail: tbuha@ciimar.up.pt

Current methods for assessing immune and health status often rely on invasive procedures such as tissue collection and biosensor surgical implantation. Established welfare indicators are often observed too late to enable timely intervention, and remain limited for many farmed species. The skin and skin mucus of teleost fish play crucial roles in both innate and adaptive immunity. Stress from common aquaculture practices, such as transport, can disrupt skin function, delay healing, and weaken immunity. Because of its role in maintaining homeostasis and the potential of minimally or non-invasive sampling, skin and skin mucus are promising sources for biomarker discovery, enabling earlier and less intrusive health monitoring in farmed fish. To investigate the molecular signatures following acute stress, three key European aquaculture species: Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*) were submitted to transport-induced acute stress. The transportation trials were designed to simulate current transport conditions specific to each species. Skin tissue and mucus samples were collected at three time points: before transport, immediately after stress exposure, and following a 24-hour recovery period. RNA sequencing was conducted on skin samples, while miRNA and proteomic analyses were performed on skin mucus, followed by differential expression analysis. Results indicate that the stress response was species specific with different timings and magnitudes of response. Comparative analysis revealed shared genes, miRNAs and proteins. Among the commonly differentially expressed elements, candidate biomarkers with prominent roles in coagulation, wound healing and immunity were highlighted.

**Keywords**: Aquaculture, Biomarkers, Multiomics, Stress, Transport

## Session 7 — Reproduction & improved breeding I

## Chairs: Dean Jerry, Renan Appel

### PRECISION GENOMICS IN ARCTIC CHARR: CUSTOM SNP-CHIPS FOR BACTERIAL KIDNEY DISEASE RESISTANCE AND DIVERSITY MANAGEMENT

Palaiokostas, C.[1] and Johnsson, M.[1]

[1]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden*

e-mail: christos.palaiokostas@slu.se

Arctic charr (*Salvelinus alpinus*) is a high-value salmonid for Nordic aquaculture. A national breeding program for Arctic charr has been operating in Sweden for over 40 years, focusing primarily on growth-related traits and relying on traditional pedigree-based selection. Notably, no trait related to disease resistance has been included in the program's breeding goal previously. Two SNP arrays containing 600,000 and 72,000 markers, tailored for selectively bred Arctic charr, were developed and used to study genetic diversity and the potential to select for charr resistant to bacterial kidney disease (BKD), respectively. Initially, the high-density array was applied to a representative sample of the breeding nucleus to gain insights into the status of genetic diversity. Runs of homozygosity (ROH) longer than 4 Mbp, suggested for a median inbreeding coefficient of approximately 9%, while at the same time indicating the existence of an island of homozygosity in chromosome 8 (NCBI: GCF_045679555.1). Genotyping with the 72k array of approximately 2,000 juveniles from the 2025 year-class that had undergone a BKD challenge experiment indicated substantial potential to select for resistant charr, as moderate-to-high heritabilities were obtained (~0.46-0.58). Genome-wide association analysis (GWAS) did not detect any quantitative trait loci of moderate to large effect suggesting that BKD resistance resembles a polygenic trait. Furthermore, genomic selection practices further supported the potential of breeding for BKD resistant Arctic charr. Overall, the development of the SNP-arrays is expected to boost the Swedish Arctic charr breeding program allowing for more efficient selection decisions.

**Keywords:** Arctic charr, bacterial kidney disease, SNP-array, genomic selection

# COMPLEXITIES OF SELECTIVE BREEDING WITHIN PEARL OYSTERS – A LONGDITUDINAL STUDY REVEALS MOLECULAR DRIVERS OF SHELL GROWTH AND COLOUR

Jones, D.B. [1], Massault, C. [1], Zenger, K.R. [1] and Jerry, D.R. [1]

e-mail: david.jones3@jcu.edu.au

Pearl production in Australia has been of substantial economic and cultural importance since the early twentieth century. Despite decades of optimization of husbandry and culture practices, pearl production remains a biologically complex, multi-year process involving oyster grow-out, seeding, pearl deposition, harvest, and grading. This complex production system has historically limited the implementation of structured selective breeding programs.

Here, we present genetic patterns observed within a multi-cohort, individually traced dataset derived from commercial *Pinctada maxima* operations spanning approximately six years of production. Oysters were tracked from spawning and grow-out through seeding, pearl growth, harvest, and grading, enabling longitudinal analyses of traits relevant to breeding program design. Recorded traits included shell dimensions and weight, as well as nacre colour dynamics measured at three timepoints (18, 29, and 54 months).

Shell dimension traits (antero-posterior width, dorso-ventral height, and depth) exhibited asymptotic growth trajectories, while shell weight followed a sigmoidal pattern, reflecting shifts in energetic allocation across ontogeny. Changes in inner shell nacre colour were strongly associated with subsequent growth trajectories, suggesting that nacre colour may act as a correlated indicator of growth performance.

Moderate heritabilities were observed for key shell growth traits, demonstrating substantial additive genetic variation and confirming that growth performance can respond effectively to selection. Inner shell nacre colour traits also showed measurable heritability and considering inner shell colour is correlated with the colour of the pearl produced, it also places it as a likely target for selection.

However, genetic correlation analyses revealed an antagonistic relationship between growth and silver or white inner nacre colour. Oysters expressing silver nacre tended to exhibit slower growth, whereas yellow nacre was associated with faster growth. This negative genetic correlation suggests that simultaneous improvement of both traits within a single breeding objective may be constrained. These findings support the implementation of a two-line breeding strategy in *P. maxima*, with host lines selected for growth performance and donor lines selected for desirable nacre colour traits that influence pearl colour outcomes.

**Keywords**: Aquaculture, Pearl oyster, *Pinctada maxima*, Selective breeding, shell colour

# THE SPERM RACE MATTERS: PHENOTYPIC AND GENOMIC EVIDENCE FOR WITHIN-EJACULATE HAPLOID SELECTION IN *DICENTRARCHUS LABRAX*

Allal, F.[1], Delpuech, E. [1], Barbarosoglu, S.-N.[1], Vergnet, A.[1], Sánchez-Baizán, N.[1] and Vandeputte, M.[1]

[1]*MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, INRAE, Palavas-les-Flots, France*

e-mail: Francois.Allal@ifremer.fr

Does the sperm race matter for offspring quality? Haplotype Selection Theory predicts that within-ejaculate sperm competition acts as a pre-fertilization genetic filter, with sperm phenotype partly reflecting the haploid genotype carried. We tested this in European seabass by applying three pre-activation delays (0 s, 30 s, 60 s) between sperm activation and fertilization across 2 replicate tanks per treatment, imposing a longevity-based selection pressure within a single ejaculate. A total of 839 offspring from 9 maternal half-sib families (28–202 individuals per family) were genotyped with a SNP array (11,546 informative markers). Paternal allele transmission was phased across linkage groups. Dam, treatment, replicate nested within treatment, and dam × treatment interaction effects were tested sequentially via likelihood-ratio tests. Genome-wide significance was assessed by permutation across individuals. Phenotypically, the 30 s delay produced higher survival (36% vs 25%; $P < 0.0001$) and earlier swim bladder inflation (56% vs 21% at 14 dpf; $P < 0.001$). Genomically, the dam effect dominated allele transmission variation (Fig. 1). After controlling for dam and replicate effects, the treatment effect showed suggestive localized signals on LG2 and LG7 (Fig. 1), while the dam × treatment interaction revealed additional locus-specific heterogeneity on LG2 and LG9 (Fig. 1, bottom panel). Neither the treatment nor the replicate effect reached genome-wide significance after FDR correction, though permutation-based analyses are ongoing. The phenotypic advantage of the 30 s condition and the suggestive genomic signals open a compelling perspective: a simple pre-activation delay at fertilization may be sufficient to reshape the allelic composition of a cohort. Demonstrating this conclusively at the genomic level likely requires larger family sizes and a higher number of informative meioses than available in the current design.

**Keywords:** haploid selection, sperm pre-activation, transmission ratio distortion, replicate effect, *Dicentrarchus labrax.*

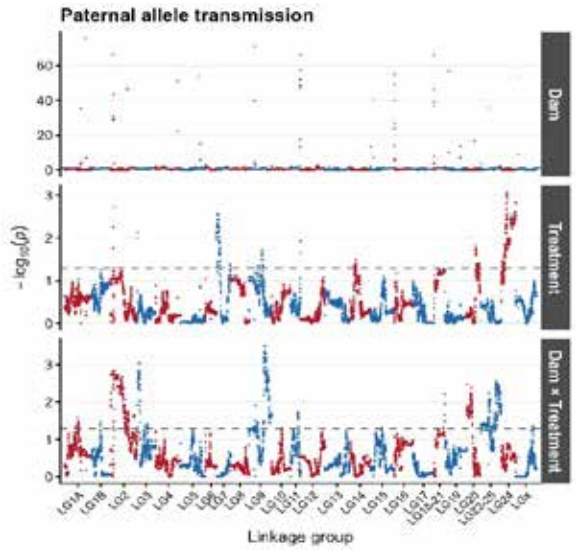



***Figure 1.*** *Genome-wide analysis of paternal allele transmission distortion. Dots represents SNP markers ordered by linkage group along the x-axis. The y-axis shows -log10(p) from sequential likelihood-ratio tests on allele transmission frequency. Top: dam effect. Middle: treatment effect, estimated after controlling for dam and replicate effects. Bottom: dam × treatment interaction. Dashed line: nominal significance threshold (p = 0.05). Permutation-based genome-wide thresholds are pending.*

# TRANSCRIPTOMIC AND EPIGENETIC CHANGES IN THE OLFACTORY ORGANS OF SENEGALESE SOLE MAY UNDERLIE REPRODUCTIVE FAILURE IN CAPTIVE-BRED MALES

Torres-Sabino, D. [1], Pereira, A.L. [1], Carballeda, M. [1], Aramburu, O. [1], Robledo, D. [1,2], Bouza, C. [1] and Martínez, P. [1]

*1Department of Zoology Genetics and Physical Anthropology, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain, 2The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK*

e-mail: dorinda.torres.sabino@usc.es

Senegalese sole (*Solea senegalensis*) is a promising flatfish species for European aquaculture. However, its production faces significant challenges, primarily the incapacity of captive-bred males (CB) to perform courtship behavior and reproduce, unlike their wild-bred (WB) counterparts. This issue is hypothesized to stem on epigenetic modifications of genes involved in chemical communication mediated by the olfactory system. Flatfish possess an upper olfactory organ exposed to the water column and associated with intra-specific chemical communication, whereas the lower olfactory organ is in contact with the substrate and involved in food detection. Various approaches have been developed to elucidate the genetic foundation of this crucial yet understudied sensory system in *S. senegalensis*, critical for social and environmental interactions. In this work, we examined the transcriptomic profiles of the upper and lower olfactory organs of *S. senegalensis* CB and WB males using RNA-seq. Different life-histories (CB *vs.* WB) linked to different environmental factors could affect the epigenetic mechanisms regulating gene expression, such as DNA methylation. To check this hypothesis, we performed EM-seq to compare the methylation profiles of the olfactory rosettes. A list of differentially expressed genes (DEGs) and differentially methylated genes (DMGs) across four comparisons involving the upper and lower rosettes of CB and WB males was generated. Furthermore, the integration of both analyses detected genes that were differentially expressed and methylated (DEMGs), including olfactory receptor genes responsible of the detection of chemical cues and others associated with hormone signalling and reproduction. Notably, CB males exhibited more similar transcriptomic profiles, likely reflecting their lifelong exposure to uniform captive environmental conditions. Interestingly, the strongest transcriptomic and epigenetic disparities were found when comparing the upper rosettes of CB *vs.* WB males. These results highlight the influence that early-life environment and prolonged exposure play in the regulation of gene expression, possibly linked to the different reproductive capacities of *S. senegalensis* males.

**Keywords**: EM-seq, RNA-Seq, reproduction, chemical communication, Senegalese sole

# OVULATION DEFINES FINAL MATERNAL RNA LANDSCAPE THROUGH EXTENSIVE TRANSCRIPTOMIC REMODELING IN PIKEPERCH EGGS

Żarski, D.[1], Nynca, J.[1], Ljubobratović, U.[2], Klopp, C.[3], Palińska-Żarska, K.[4] and Bobe, J.[5]

*[1]InLife Institute, Poland; [2]MATE, Hungary; [3]INRAE Toulouse, France; [4]IFI Olsztyn, Poland; [5]INRAE Rennes, France*

e-mail: d.zarski@pan.olsztyn.pl

Maternal transcripts govern early embryogenesis in fish, yet the timing of final maternal RNA landscape formation during oogenesis remains unclear. Here, we performed time-resolved, oocyte-specific RNA-seq in pikeperch (*Sander lucioperca*) using repeated intra-individual sampling, enabling resolution of transcriptomic dynamics independent of inter-female variability. The maternal transcriptome remained largely stable from late vitellogenesis to the onset of final oocyte maturation (FOM), followed by extensive remodeling at ovulation. More than 8,000 genes changed in abundance exclusively during the transition to ovulated eggs, whereas earlier stages showed minimal variation. Ovulated eggs were enriched in transcripts associated with RNA processing, cytoskeletal organization, and cell-cycle regulation, alongside depletion of translation- and ribosome-related genes. As these changes occur during a transcriptionally quiescent stage, they are unlikely to reflect de novo transcription alone and are consistent with extensive post-transcriptional regulation. Ovulation-associated transcripts strongly overlapped with egg-specific genes and candidate egg-quality markers, defining a core set of maternal-effect candidates. These results indicate that maternal RNA provisioning is largely finalized at ovulation, identifying this stage as a critical window for egg-quality determination and biomarker discovery in aquaculture.

**Keywords**: RNA-seq, oocyte maturation, maternal transcripts, egg quality, pikeperch

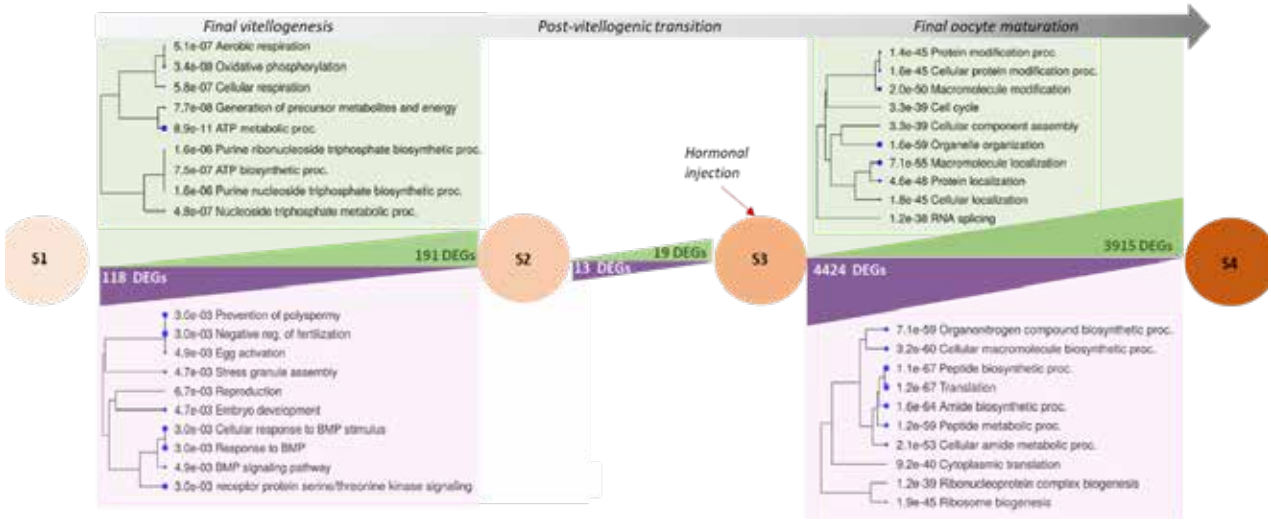



***Figure 1.*** *GO analysis of DEGs between different (S1-S3) stages of oocyte maturation and ovulated eggs (S4). S1 – late vitellogenesis, S2 – end of vitellogenesis, S3 – onset of FOM, and S4 – ovulated eggs.*

# TRANSCRIPTOMICS OF RAINBOW TROUT EGGS USED FOR GYNOGENESIS: PROTECTION FROM PHYSICAL STRESS VS. SENSITIVITY OF MICROTUBULES FOR HYDROSTATIC PRESSURE SHOCK

Ocalewicz, K. [1], Kuciński, M.[1], Pałucha, K. [1], Panasiak, L. [1] and Rożyński R.[2]

[1] *Faculty of Oceanography and Geography, University of Gdansk, Poland,* [2]*Department of Salmonid Research, Inland Fisheries Institute in Olsztyn, Poland*

e-mail: konrad.ocalewicz@ug.edu.pl

Gynogenesis is a reproductive mode where offspring inherit exclusively maternal chromosomes. Gynogenetic development in fish may be induced intentionally by activating eggs with the UV-irradiated spermatozoa. The resultant haploid gynogenetic zygote is then exposed to a physical shock to prevent release of the 2nd polar body or to inhibit 1$^{st}$ cell cleavage to reconstitute diploid state of the embryo and to generate meiotic and mitotic gynogenotes, respectively. We have observed that rainbow trout eggs originated from different females differ in the predisposition for gynogenesis and this characteristic is not always related to the egg quality sensu stricto (developmental competence). Eggs with the highest developmental potential for gynogenesis showed increased transcription of genes involved in the oocyte maturation (*Rasl*11b) and protection from the physical stress (*Keratin* 8), apoptosis (*Caspase* 6, *Pgam*5) and early embryogenesis including maternal to zygotic transition (*Gata*2) and decreased abundance of *Keratin* 18 and microtubule related *Tubulin* β mRNA transcripts. As gynogenetic development may be impaired by UV-induced chromosome fragments (improper sperm inactivation) and ploidy disturbances (haploid/diploid mosaicism), efficiently acting apoptosis enabled better regulation of the early embryonic development and decreased early mortality among gynogenetic rainbow trout. Moreover, since keratins protect oocytes from physical stress after ovulation, the high abundance of *Keratin 8* in the rainbow trout eggs may increase their resilience to the physical shock applied for the zygote diploidization during gynogenesis. On the other hand, a low level of tubulin-building microtubules may increase the efficiency of high hydrostatic pressure (HHP) shock used for diploidization of the gynogenetic zygotes.

**Keywords**: Gynogenesis, Egg quality, Maternal RNA,

***Reference***

K. Ocalewicz et al. (2025). Genes *16*(7), 803

K. Ocalewicz et al. (2025). Fishes *10*(11), 585

## Session 8 — Reproduction & improved breeding II

## **Chairs:** Elena Sarropoulou, Ivana Buselic

### ROOTING THE FUTURE OF SPACE AQUACULTURE

Ribas, L. [1], Przybyla, C. [2], Mehta, T. [3], Hernández, R. [4], Peña, C. [4], Mistsopoulou, M. [1] and Joly, S. [1]

*[1]Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain; [2]French Institute for Ocean Science (IFREMER), Palavas, France; [3]Institute of Systems, Molecular and Integrative Biology, University of Liverpool, United Kingdom; [4]Laboratorio Subterráneo de Canfranc (LSC), Canfranc-Estación, Spain.*

e-mail: lribas@icm.csic.es

Longduration human space missions require resilient, autonomous food production systems capable of sustaining crew health beyond Earth. Space aquaculture has emerged as a promising way to produce highquality animal protein in extraterrestrial environments. Within this context, we present preliminary data in spacesimulated environments combining developmental biology, environmental stress physiology, transcriptomics and epigenetics to study how spacerelated constraints affect early development in two key fish species: zebrafish (*Danio rerio*) and European sea bass (*Dicentrarchus labrax*). Here, we show early findings on how the combined effects of microgravity and cosmic silence influence survival and behavioural traits and compared to background radiation. Using sequencingbased approaches, we focus on identifying transcriptomic and epigenetic signatures that reflect early stress responses and developmental plasticity. Initial analyses show high survival in both simulated microgravity and cosmic silence conditions, being fish swimming behaviour clearly altered under microgravitylike environments. Sequencing data from both the transcriptome and methylome reveal a limited number of genes altered both in the expression and in the methylation. These mainly involve pathways related to DNA maintenance, developmental regulation, immune system and tissue repair. This apparent modest physiological and molecular response suggests that fish embryos have a notable capacity to tolerate environmental conditions that mimic spacerelated stress. Such resilience offers valuable guidance for the future design of spacebased aquaculture systems and for breeding strategies aimed at producing fish phenotypes able to cope with unpredictable climatic challenges on Earth. Overall, our pilot experiments provide an encouraging sign for the longterm feasibility of aquaculture beyond our planet.

**Keywords**: transcriptome, methylome, space, early development

***Figure 1.*** *AIgenerated visualization illustrating a conceptual space aquaculture system.*

# SHARED GENETIC ARCHITECTURE REVEALS PLEIOTROPIC QTL(S) REGULATING GONADOSOMATIC INDEX AND SEXUAL MATURITY IN ATLANTIC COD

Aslam, M.L. [1], Kettunen, A.H. [1 2], Hansen, Ø.[1] Fernandes, J.M.O.[3], Konstantinidis, I.[4] and Lillehammer, M. [1]

[1]*Nofima AS, Ås, Norway;* [2]*Nordic Genetic Resources Center, Ås, Norway;* [3]*Institut de Ciències del Mar, CSIC, Barcelona, Spain;* [4]*Nord University, Bodø, Norway*

e-mail: luqman.aslam@nofima.no

Puberty in Atlantic cod diverts metabolic resources from somatic growth toward gonadal development, accompanied by morphological and behavioural changes that reduce production efficiency. Although photoperiod and temperature influence maturation timing, their consistent application in large sea cage systems is limited. Pubertal onset reflects the integration of environmental cues with a genetically regulated framework, and individuals differ in their heritable sensitivity to these stimuli. To characterize the genetic architecture underlying sexual maturation, approximately 2,467 individuals from the Norwegian National Cod Breeding Program representing 168 families from year-classes 2019 and 2023 were phenotyped. Maturation status (SM) was assessed via gonadal inspection, and the gonadosomatic index (GSI) was calculated to quantify reproductive investment. All fish were genotyped using the GGP Illumina Infinium™ array (~21K SNPs). In parallel, whole genome sequence data from 111 individuals (Research Council of Norway project #207680) enabled Beagle 5.4 imputation, yielding high density variant datasets. Quantitative genetic analyses revealed moderate to high heritability for SM and GSI and a strong genetic correlation between them. Genome-wide association and fine mapping detected overlapping QTLs with concordant allelic effects, supporting a pleiotropic genetic basis for both traits. The QTL regions (±250 kb) for SM contained numerous genes (Figure 1), with inpp5b, acvr1c, and igf2r emerging as key functional candidates due to their documented roles in gonadal development and growth regulation. Depending on the analytical method, the four lead SNPs (one from each QTL) collectively explained up to ~29% of the total genetic variance. These genetic findings will be integrated with epigenetic signatures of maturation generated in EPICOD (Research Council of Norway project # 336112).

**Keywords**: SNPs, QTL, GWAS, Fine-mapping, Heritability, Sexual Maturation

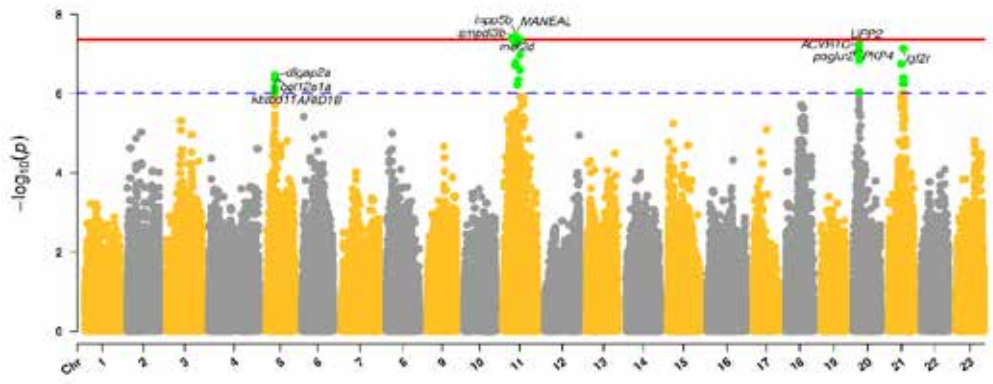



***Figure 1.*** *Manhattan plot for sexual maturation shows the QTL signals and candidate genes.*

# REPRODUCTIVE PERFORMANCE OF ZEBRAFISH (*DANIO RERIO*) CRISPR/CAS9 MUTANT LINES

Mengesha, T.[1], Pavlids, M.[2] and Mylonas, C.[3]

[1]*University of Gondar,* [2]*University of Crete,* [3]*Hellenic Center for Marine Research*

email: mebratuaw@gmail.com

Zebrafish (*Danio rerio*) is a useful animal model in which to examine the function of glucocorticoid (GR) and mineralocorticoid (MR) receptors in the reproductive performance of teleost. The present study aimed at examining the reproductive performance of zebrafish lines, and in particular wild type (WT) and CRISPR/Cas9- generated GR and MR knockouts (*gr*-/-, *mr*-/-), under pair and mass spawning conditions. Outcomes were assessed, spawning performance (egg production, fertilization success, survival of embryo, early and mid-larva stage), sperm quality (using CASA technology) and reproductive status (through gonadal histology). A total of 60 adult zebrafish (20 fish/genotype, even sex distribution) were held in standardized conditions. The $gr^{-}$ $/^{-}$ line was unable to reproduce through pair spawning in repeated trials however it was successfully maintained under mass- spawning conditions. *mr*-/- zebrafish displayed markedly higher fertilization (94.97%) and cumulative survival rates (71.43%) than wildtype (70.87%, 35%, $p = 0.016, 0.013$, respectively) during pair- spawning. In contrast, survival at 3dpf in mass spawning of gr-/- larvae was significantly reduced ($p = 0.011$). Larval growth was almost age-dependent in both pair and mass spawning (Adjusted $R^2 = 0.839$; $p < 2 \times 10^{-16}$). Mean length grew from 1.27 at 3dpf to 1.80 at 28dpf. Genotype effects were not significant in general, but for mass spawners $mr^{-}$ $/^{-}$ and $gr^{-}$ $/^{-}$ larvae exhibited weak (age-dependent) differences ($p < 0.0006$). No statistically significance differences among the different genetic lines on sperm motility were observed, although mr-/- males had a 28% reduced motility. Histological examination revealed robust gonadal dysgenesis in mr-/- fish, consisting of oocyte arrest and disrupted spermatogenesis, whereas *gr*-/- females laid larger eggs (0.75mm, $p < 0.05$). This work contributes to better understanding of corticosteroid signaling in fish reproduction and has application to aquaculture and biomedical research.

**Keywords:** CASA, glucocorticoid receptor, mineralocorticoid receptor, spawning, zebrafish

## Session 8 — Low trophic aquaculture

## **Chairs:** Elena Sarropoulou, Ivana Buselic

### EXPLORING GENETIC DIVERSITY AND HYBRIDISATION PATTERNS OF MUSSELS IN NORTHERN SCOTLAND AND IRELAND

Chapuis, A[1].; Simon, A[2].; Cariolato, E[3].; Mirimin, L.[3]; Ellis, R.[4] and Regan, T[1].

*[1]The Roslin Institute, Edinburgh, Scotland; [2] CNRS-Sorbonne Université, Station Biologique de Roscoff, France; [3]Marine and Freshwater Research Centre, Atlantic Technological University, Galway, Ireland; [4]College of Life and Environmental Sciences, University of Exeter, UK*

e-mail: achapuis@ed.ac.uk

Understanding genetic diversity and gene flow within and between mussel populations informs population fitness and contributes towards aquaculture sustainability. Shetland, in the north of Scotland, is responsible for >60% of UK blue mussel production. As such, we aimed to investigate the genetic diversity and population structure of mussels grown in this important location compared with populations elsewhere in the north of Scotland. DNA was isolated from samples taken from four populations, each containing 30 animals: two from northeast mainland Scotland (Cromarty Firth); one from northwest of Scotland (Western Isles); and two populations of locally adapted Shetland mussels. Single Nucleotide Polymorphisms (SNPs) analysis was performed using a medium density multi-species *Mytilus* array. To analyse subspecies hybridisation, we also included reference samples of *Mytilus* subspecies: *M. galloprovincialis, M. edulis* and *M. trossulus.* The initial analysis reveals that Cromarty populations appear to predominantly have *M. edulis* genetics. On the other hand, mussels from Shetland and the Western Isles display levels of introgression with *M. galloprovincialis.* Higher levels of genetic conservation were seen in the Western Isles compared with the relatively diverse genetics observed in both Shetland populations. Further analysis of each population using Nanopore sequencing was carried out to explore the levels of large structural variation (SV) and Presence/Absence Variations (PAV) in stress-response genes. Taken together, these data demonstrate that this SNP array provided a robust platform for consistent genotyping of individuals and may be used to further investigate appropriate growing environments for background genetics to enhance mussel health and productivity.

**Keywords**: Mussels, SNPs, Genotyping, Population

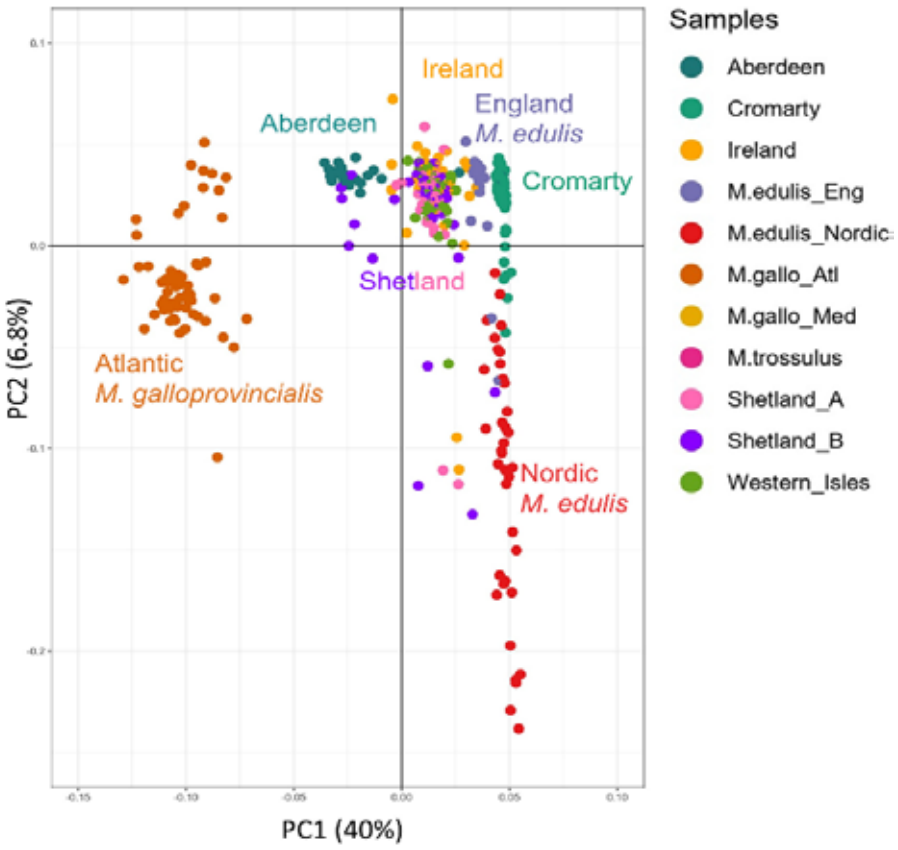



***Figure 1:*** *Population structure of European Mytilus samples based on geographic origin and principal component analysis.*

### *Reference*

Chapuis, A. F., Simon, A., Arthur, G., Fraslin, C., Cariolato, E., Mirimin, L., Ellis, R. P., & Regan, T. (2026). *Aquaculture*, *615*.

# LONG-READ SEQUENCING REVEALS EXTENSIVE STRUCTURAL VARIATION IN FARMED SCOTTISH *MYTILUS* MUSSELS

Chapuis, A.F.[1], Gundappa, M.K.[2], Valk, S.[2] and Regan, T.[1]

[1]*The Roslin Institute, University of Edinburgh, UK,* [2]*Animal Breeding and Genomics, Wageningen University and Research, Netherlands*

e-mail: tim.regan@roslin.ed.ac.uk

Marine mussels of the genus *Mytilus* exhibit high genetic diversity and frequent hybridisation, yet most population genomic studies rely on SNP markers that capture only a fraction of genomic variation. Structural variants (SVs), including large insertions and deletions, may represent a substantial but underexplored component of diversity in these species. To place structural variation in a population genomic context, we first analysed 200 Scottish mussels genotyped using the 60K MytiSNP array. Principal component analysis revealed clear species and hybrid structure across five populations. Four Scottish individuals representing maximally divergent genotypes were selected for long-read sequencing. High molecular weight DNA was sequenced on the Oxford Nanopore PromethION platform to ~30-70× coverage per individual. Libraries were prepared from DNA fragmented to ~20 kb, producing reads with an N50 of ~14 kb. Error-corrected reads were analysed using a structural variant detection pipeline, with consensus calls retained across three SV callers: SVIM, cuteSV and Sniffles. Across the four genomes we identified structural variation including thousands of insertions and deletions larger than 1 kb and many very large variants reaching tens of megabases in length. These results are consistent with recent *Mytilus* genome and pangenome studies indicating highly dynamic genome architecture. Although based on a limited number of individuals, this pilot study highlights the scale of structural genome diversity present within Scottish farmed *Mytilus* populations and demonstrates the utility of long-read sequencing for resolving genomic variation largely inaccessible to SNP-based approaches.

**Keywords**: Genomics, Long-read, ONT, Structural variation, Pangenome, Bivalve, Mussel

# FROM CHALLENGE TEST TO GWAS: UNCOVERING GENOMIC SIGNALS OF SALINITY TOLERANCE IN THE COMMON COCKLE

Gallo, M.[1§], Cerviño-Otero, A.[2], Hermida, M.[3], Nóvoa, S.[2], Túñez, A.[2], Robledo, D. [1,4], Martínez, P.[3]

*[1]Department of Zoology, Genetics and Physical Anthropology, Biological Research Center (CIBUS), University of Santiago de Compostela, Santiago de Compostela, Spain; [2]Centro de Investigaciones Marinas (CIMA), Consellería do mar – Xunta de Galicia, Corón, Pontevedra, Spain; [3]Departament of Zoology, Genetics and Physical Anthropology, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain; [4]The Roslin Institute, Edinburgh, Scotland.*

e-mail: marialaura.gallo1@usc.es

Climate-driven environmental changes are increasing the frequency of extreme freshwater inputs in estuarine ecosystems, resulting in abrupt salinity drops that cause mass mortality events in bivalve populations. In Galicia (NW Spain), the common cockle, *Cerastoderma edule*, is a key aquaculture and fishery resource whose production is increasingly threatened by these episodic salinity shocks.

This study aims to investigate the genetic architecture of salinity tolerance in *C. edule* using a genomic approach under controlled challenge experiments. A large cohort of approximately 4,000 individuals derived from a wild broodstock was exposed to acute salinity stress under extreme (0 ppt) and edge-limit (13 ppt) conditions. Resistance (binary survival) and tolerance (time to death) were recorded as phenotypic traits. Individuals were genotyped using a 2bRAD sequencing approach, generating ~90,000 genome-wide SNP markers for downstream genomic analyses. Genome-wide association studies (GWAS) were conducted to identify genomic regions associated with variation in salinity tolerance. The analyses revealed moderate and significant heritability for salinity tolerance ($h^2 = 0.31$) and identified several SNP markers significantly associated with survival-related phenotypes across several chromosomes, suggesting a polygenic architecture underlying the response to salinity stress.

To further explore the interaction between environmental stressors, a second experiment was established to assess the combined effects of salinity, temperature and size across different life stages. Individuals from three size classes (8 mm, 13 mm, and adults) were exposed to a factorial combination of four temperatures and five salinity conditions, and mortality dynamics were monitored through survival curves.

Together, these studies provide genomic and biological insights into the adaptive response of *C. edule* to salinity stress and represent a step toward integrating genomic information into breeding and management strategies aimed at improving the resilience of cockle aquaculture under future climate scenarios.

**Keywords**: *Cerastoderma edule,* salinity tolerance, GWAS, Genomic selection

# POSTER PRESENTATIONS

## Posters — Immunity, stress & welfare

### FUNCTIONAL VALIDATION OF IFI27L2A AS A RESTRICTION FACTOR FOR NNV REPLICATION

Arana, A.J.[1,*], Polo, D.[2], Costa, A.[2], Cuesta, A.[3], Martínez, P.[1], Robledo, D.[1,4] and Sánchez, L.[1]

*[1]AQUIGEN Group (GI-1251), Faculty of Veterinary Medicine, Campus Terra, Universidade de Santiago de Compostela, 27002 Lugo, Spain, [2]NANOTOXGEN Research Group (G000806), CICA-Faculty of Science, Universidade da Coruña, 15071 A Coruña, Spain, [3]Fish Innate Immune System Group (UM Group 31871), Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain, [4]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Edinburgh EH25 9RG, UK*

*Corresponding contact: alvaro.arana@usc.es

Viral nervous necrosis (VNN; betanodavirus) causes major losses in European seabass (*Dicentrarchus labrax*) and affects gilthead seabream (Sparus aurata), and resistance shows exploitable genetic variation in farmed populations. Notably, recent population-genomic and integrative omics studies in seabass have highlighted IFI27-like genes, including the teleost orthologue ifi27l2a, as candidates linked to VNN outcome. Functional validation, however, is often limited because many seabass/seabream cell lines are poorly permissive to VNN in vitro, complicating the study of host determinants. Here, we tested the antiviral role of ifi27l2a using genome-edited (ifi27l2a−/−) and wild-type (WT) derivatives of seabass (DLB-1) and seabream (SaB-1) cell lines. Cells were infected with VNN and viral RNA was quantified by absolute RT-qPCR in intracellular lysates and culture supernatants across a time course (1–168 h post infection), using the highly permissive E-11 line (standard for VNN propagation/production) as a positive control. As expected, E-11 showed massive replication (~$10^{10}$ copies at 24 h) with marked cytopathic effect, whereas DLB-1 and SaB-1 WT displayed restricted replication (typically $10^6$–$10^7$ copies, only 1 LOG increase in viral RNA levels from the initial load) and no clear CPE. Loss of ifi27l2a increased replication predominantly intracellular: in DLB-1, mutants showed early increases versus WT (~5.0× at 12 h and ~3.2× at 24 h, intracellular), while in SaB-1 the effect was stronger, peaking at 24 h (~21.1× intracellular and ~9.0× in supernatant), consistent with enhanced production and release. Overall, these results support ifi27l2a as a restriction factor limiting VNN replication in seabass and seabream cells, bridging population-genomic signals with mechanistic validation and strengthening in vitro platforms for host-determinant discovery and antiviral screening.

**Keywords:** Fish cell lines; CRISPR; Susceptibility; Host defense; Functional genomics

# VITTELOGENINS EXCLUSION DURING MASS SPECTROMETRY DATA ACQUISITION ENHANCES PROTEIN CO-EXPRESSION ANALYSIS IN EGGS FROM PATHOGEN CHALLENGED ZEBRAFISH

Bastos, B.[1], Molés, G.[1], Hinzmann, M.[1], Costas, B.[1] and Rocha, A.[1]

[1]*CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto.*

e-mail: bbastos@ciimar.up.pt

Quantitative protein profiling of zebrafish (*Danio rerio*) eggs is hindered by the high abundance of yolk proteins, which can mask less abundant yet biologically relevant proteins. Conventional approaches have attempted to reduce the amount of yolk in egg protein extracts, along with a range of mass spectrometry approaches. Nonetheless, eliminating the yolk, derived mostly from multiple types of vitellogenins (Vtgs), may inadvertently also discard proteins that are physically or biochemically similar to yolk proteins. Here, we applied a strategy that avoids yolk elimination during sample preparation by de-prioritizing Vtgs proteins identification during mass spectrometry data acquisition. This approach was used to determine if challenging zebrafish broodstock with a nonlethal dose of *Edwarsiella piscicida* prior to reproduction can improve the parental transfer of immunity. Pools of 20 eggs from challenged and unchallenged fish were collected at 1-cell stage. Only spawns with embryo survival rates exceeding 90% at 24 hours post-fertilization were considered for further analysis. Quantitative proteomic profiling was performed using tandem mass tag (TMT) isobaric labelling for accurate quantitation. Differential expression analysis following Vtgs exclusion revealed that all studied associated proteomes yielded more DEPs (holistic, ~2.2-fold; sperm, ~1.7-fold; egg, +6 and immune, +2 DEPs; $p < 0.05$; $|LFC| > 0.5$). After Vtgs exclusion from mass spectrometry data, weighted topological co-expression networks were ~1.18-fold larger and denser in infected samples, exhibiting greater change in positive rewiring ($\Delta wTO = wTO_{infected} - wTO_{control}$), with infected networks showing higher connectivity across holistic, egg-, sperm-, and immune- associated proteomes. Differential co-expression analysis recovered more specific (γ) nodes in infected networks, indicating broader structural reorganization during infection response. Overall, Vtgs exclusion during TMT LC-MS/MS advances Vtgs-rich workflows by preserving proteome integrity, while unmasking biologically relevant signals. This method reveals infection-driven proteome remodeling and transgenerational immune priming signatures with direct aquaculture relevance for enhancing larval disease resistance.

**Keywords**: Proteomics; Zebrafish; Immunity; Co-expression networks

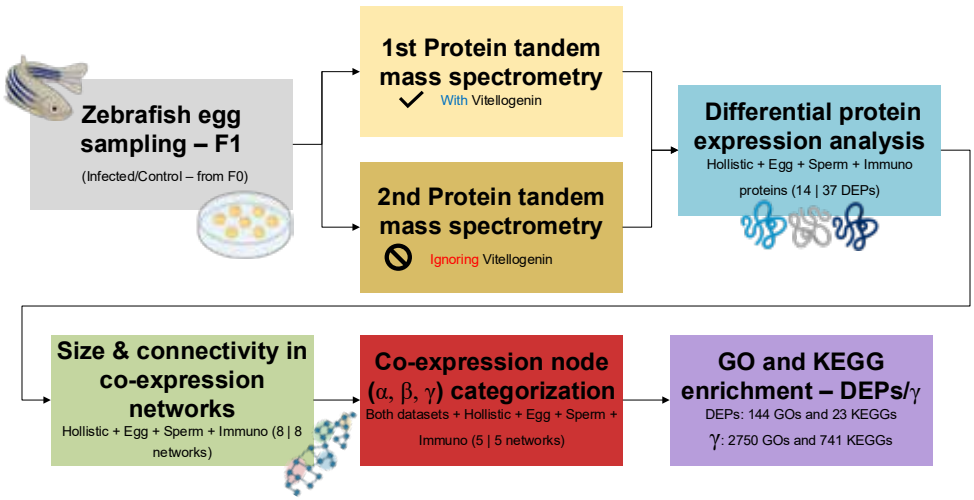



***Figure 1.*** *Methodology flowchart.*

# NUCLEOTIDE SEQUENCE BASED EPIDEMIOLOGICAL SURVEILLANCE IS ESSENTIAL FOR TRACKING VHS OUTBREAKS IN AUSTRIAN TROUT AQUACULTURE

Budik S. [1], Kaltenegger G. [2], Wachter L. [3], Bischof A. [1], Hiesel J. [1] and Vogl G. [4]

*[1]Veterinärdirektion Land Steiermark, Austria; [2]Veterinärreferat BH Leoben Land Steiermark, Austria, [3]Veterinärreferat BH Bruck-Mürzzuschlag Land Steiermark, Austria, [4]Institut für Lebensmittelsicherheit u. Veterinärwesen, Amt der Kärntner Landesregierung, Austria,*

e-mail: sven.budik@stmk.gv.at

Viral hemorrhagic septicemia (VHS) is caused by the viral hemorrhagic septicemia virus (VHSV), a rhabdovirus known for its high genetic variability and rapid evolution. VHSV is capable of infecting more than 80 fish species in marine and freshwater environments in the Northern Hemisphere causing great economic loss. Currently VHSV is classified in four Genotypes and several subtypes. However, almost all disease outbreaks in mainland Europe are caused by only two Subtypes, making epidemiology based solely on this typing system futile. In 2025, a suspected VHSV outbreak was reported to the competent authorities. Sampling of diseased fish, followed by RNA extraction and RT qPCR confirmed the presence of VHSV in this fish in high abundance. Pathological findings were in accordance with symptoms of VHS, and the case was subsequently treated as a VHSV confirmed case by competent authorities. The epidemiological investigations did not lead to identification of any possible source of the viral infection. Currently, mainly due to lack of other evidence, the most likely introduction route might be fish predators (herons or otter). There is no rock solid evidence for that either, however. Typing of the virus as mentioned above, is not discriminative enough for the purpose of connecting the infection to prior outbreaks in Austria and bordering countries. In order to gain additional information about the origin of the introduction of the VHSV into the affected aquaculture, further differentiation of the virus by Sanger sequencing of discriminative genomic regions and BLAST based comparison to known VHSV isolates will be carried out. Patho-anatomical examination of the fish was carried out in accordance to standard protocols. For RT qPCR, head kidney was sampled and viral RNA was extracted with the Qiagen viral mini Kit. Purified RNA was analysed by RT qPCR based on the protocol by Jonstrup et al. Further typing was conducted using the RT PCR by Kahns et al. Additionally, PCR based amplification of discriminative genomic regions is carried out, and the PCR products are Sanger sequenced. BLAST anlignment of the results with NCBI and other databases are then conducted. Patho-anatomical findings and RT qPCR results are in accordance with a VHS outbreak. The genotying did not result in any strong evidence for the infection source. The sequence based analyses, phylogenetic analysis and epidemiological evaluation of these results are still ongoing and will be available soon. Detailed analysis of variable VHS sequences is a potent tool to trace the origin of this significan viral disease in order to take preventive measures by fish farmers and competent authorities likewise.

**Keywords**: Viral hemorrhagic septicemia (VHS), virus variability, epidemiology

### *Reference*

Kahns et al. 2012 Dis Aquat Org Vol. 99: 23–35, 2012 European freshwater VHSV genotype Ia isolates divide into two distinct subpopulations doi: 10.3354/dao02444

Jonstrup et al. 2013: Journal fo Fish Diseases Vol. 36: 9-23 Development and validation of a novel Taqman-based real-time RT-PCR assay suitable for demonstrating freedom from viral hemorrhagic septicaemia virus. doi: 10.1111/j.1365-2761.2012.01416.x.

# VACCINATION AT DIFFERENT LIFE STAGES DETERMINES THE IMMUNE RESPONSE TO *PISCIRICKETTSIA SALMONIS* IN ATLANTIC SALMON: A TRANSCRIPTOME ANALYSIS

Casuso, A. [1,2], Valenzuela-Muñoz, V. [1,2] and Gallardo-Escárate, C [1]

[1]*Interdisciplinary Center for Aquaculture Research-Applied Research (INCAR[2]), Laboratory of Biotechnology and Aquatic Genomics, Department of Oceanography, Universidad de Concepción, Concepción 4030000, Chile,* [2]*Center of Biotechnology, Universidad de Concepción, Concepción, 4030000, Chile.*

e-mail: acasuso@udec.cl

Vaccination programs in the salmon aquaculture industry are typically conducted during the freshwater stage when salmon weigh approximately 20 g. In Chile, data suggest that commercially available bacterial and viral vaccines provide suboptimal protection in seawater, particularly against *Piscirickettsia salmonis*. Optimizing the vaccination period relative to the production cycle could enhance immunological protection in seawater environments. This study aimed to evaluate parr and post-smolt salmon vaccination, and its effects on transcriptome modulation during *P. salmonis* infection. Atlantic salmon were vaccinated with a commercial vaccine during the parr and post-smolt stages, at weights of 20 g and 100 g, respectively. After 800 UTAs, vaccinated salmon were challenged by intraperitoneal injection with 0.1 ml of *P. salmonis* LF89 ($1x10^9$ cells/ml), and non-infected control groups for both stages were include. At 14 days post-infection (dpi), head kidney and gill tissue samples were collected for mRNA sequencing via Illumina technology. RNAseq analysis and differentially expressed genes (DEGs) were performed, using the Atlantic salmon genome as a reference. DEGs were analyzed using GO and KEGG pathways. Additionally, a mortality curve was constructed. Post-smolt vaccinated fish showed a 5-day delay in the onset of mortality compared to the parr group. However, both groups reached 86% mortality. Parr-vaccinated fish exhibited an upregulation of immune-related genes, particularly those linked to innate immunity, such as *tlr4* and *il-1β*, suggesting early immune priming. Additionally, stress-response genes in the gills, such as *hsp70*, were more highly expressed in parr salmon, indicating vaccine-induced stress. In contrast, the post-smolt group showed a marked upregulation of pro-inflammatory cytokines, including *TNF-α*, *IL-6*, and *IL-12*. Biological processes and pathways related to adaptive immunity were highly enriched in the post-smolt group. This study demonstrate that vaccination elicits distinct transcriptomic responses in Atlantic salmon, with life-stage specific immune mechanisms activated, potentially informing future vaccine strategies to enhance disease resistance in aquaculture.

**Keywords**: Vaccines, RNA-Seq, Atlantic salmon, *Piscirickettsia salmonis*

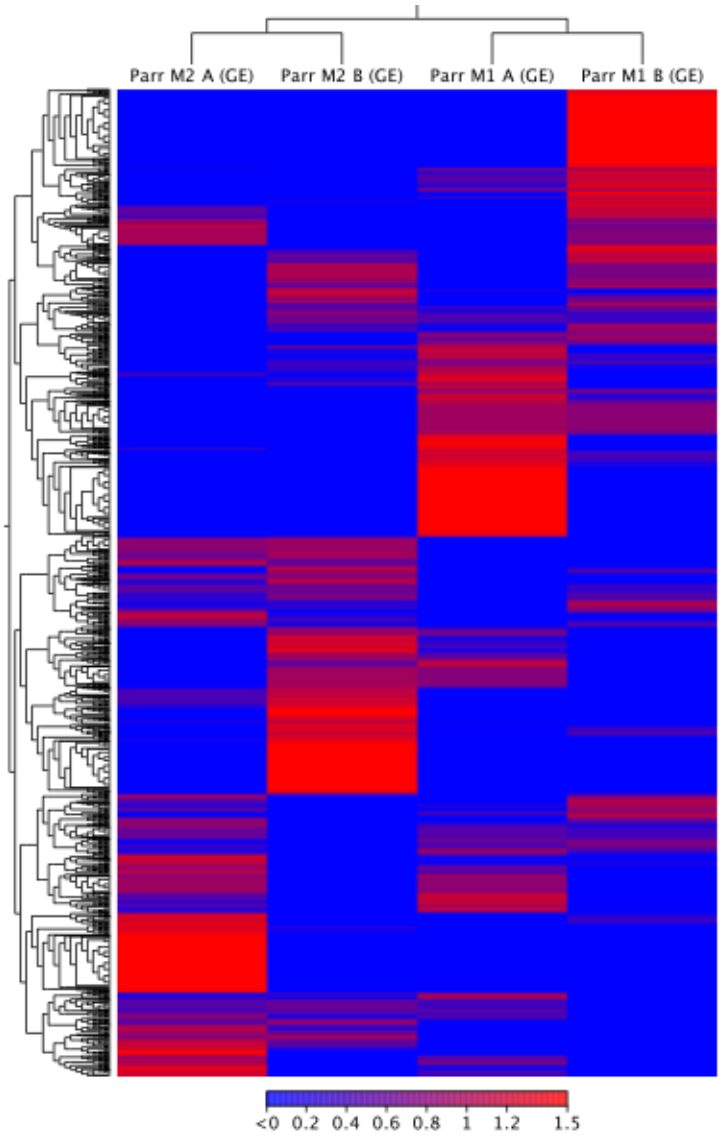



***Figure 1****. Transcriptomic profile of Atlantic salmon at the parr and post-smolt stages vaccinated and infected with P. salmonis.*

**Acknowledgments:** FONDECYT-ANID 3240484, 1210852, 11220307; CIA-ANID grant #250009.

# DIVERGENT HEALING TRAJECTORIES AMONG MECHANICAL SKIN WOUND TYPES IN POST-SMOLT ATLANTIC SALMON (*SALMO SALAR*)

Chaiyasut, K.[1], Daniels, R.R.[1], Karlsen, C.[2], Johansson, G.S.[2], Hansen, M.[2], Bou, M.[2], Bradford, B.[3] and Sveen, L.R.[2]

[1]*The Institute of Aquaculture, Stirling, Scotland;* [2]*Nofima, Ås, Norway;* [3]*The Roslin Institute, Edinburgh, Scotland*

e-mail: france.chaiyasut@stir.ac.uk

Mechanical wounds are a common form of skin damage in Atlantic salmon (*Salmo salar*) aquaculture. These injuries frequently arise from delousing treatments, handling trauma, and collisions with sea cages and other fish, especially at high stocking densities. Although several types of mechanical wounds appear similar, the healing processes underlying different forms of mechanical damage remain understudied. To improve understanding of the wound healing processes associated with different types of mechanical injury, three models were applied to post-smolt Atlantic salmon: scale loss, superficial wounds involving the epidermis and dermis, and deep wounds extending through all skin layers. Responses were assessed after two days and five weeks post-wounding to capture the inflammation and the remodelling phases of different models. These injuries were characterised primarily through targeted gene expression profiling of genes with established roles in wound healing, including *mmp9*, *col1a2*, and *cd3e*, via quantitative polymerase chain reaction (qPCR) to represent different phases. To complement the gene expression analyses, we performed histological assessment of tissue architecture, immunohistochemical evaluation of cell proliferation using an anti-phospho-Histone H3 (Ser10) (pHH3) antibody, and serum parameter analysis. Our results show that, despite their initially similar appearance, scale loss and superficial injuries have markedly different healing trajectories. Fish with scale removed returned to the pre-damage state within five weeks, whereas superficial damage elicited a delayed healing response comparable to that of deep wounds, but exhibited notable changes in hypodermis that were absent in the other models. Further investigation is required to clarify the role of the hypodermis in wound healing. These findings underscore important distinctions among wound types that should be recognised by fish health personnel and farmers, as misidentification can lead to practical setbacks such as miscalculated timing for sea lice treatments, an increased risk of secondary infections, and unexpected quality downgrades during processing.

**Keywords:** fish skin, mechanical wounds, *Salmo salar*, wound healing

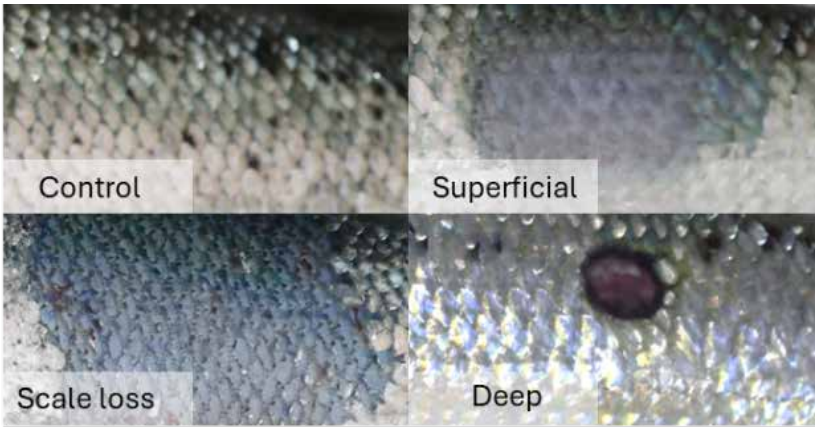



***Figure 1.*** *Overview of wound types in the experiment.*

## *Reference*

Sveen, L.R. et al. (2019). Scientific Reports, 9(1).

# POPULATION / INDIVIDUAL GENOMIC ASSOCIATION AND TRANSCRIPTOMIC DATA SUPPORTS A LONG INVERSION IN CHROMOSOME 8 UNDERLYING RESISTANCE OF THE EUROPEAN FLAT OYSTER (*OSTREA EDULIS*) TO *BONAMIA OSTREAE*

Sambade, I.M. [1], Horzli, V.[2], Bean, T.[3], Pardo, B.G.[1], Vera, M.[1], Lynch, S.[2], and Martínez, P.[1]

*[1]Universidade de Santiago de Compostela, Lugo; Spain; [2]University College Cork, Cork, Ireland; [3]Roslin Institute, Edinburgh, UK*

e-mail: paulino.martinez@usc.es

The protozoan parasite *Bonamia ostreae* has caused severe declines of the European flat oyster (*Ostrea edulis*) across the Northeast Atlantic, threatening both production areas and coastal ecosystem restoration programmes. Understanding the genetic basis of resistance is therefore essential for the development of resistant or tolerant oyster strains. Here, we investigate resistance to *B. ostreae* through a multi-layered approach integrating population genomics, structural variation, and transcriptomics.

Population genomics revealed that SNPs associated with resistance are located within a large genomic region at chromosome 8 where recombination is suppressed, consistent with the presence of a chromosomal inversion. Further analyses across the distribution range identified three major inversions in the *O. edulis* genome located on chromosomes C2, C5 and C8, collectively spanning approximately 8% of the genome. Precise mapping of C8 inversion through long-read sequencing identified genes involved in immune processes, apoptosis regulation, extracellular matrix remodelling and energy metabolism in the inversion, suggesting co-adapted alleles maintained under parasite-driven selection.

Importantly, the association between C8 and resistance was confirmed at the individual level. Oysters carrying the alternative homokaryotype were never found infected, whereas infections occurred mainly reference homokaryotypes and intermediate in heterokaryotypes, indicating an additive association model. Complementary transcriptomic analyses of naturally infected oysters revealed extensive transcriptional reprogramming along disease progression, infected individuals showing downregulation of genes involved in apoptosis, mitochondrial metabolism, adhesion and immune signalling. *B. ostreae* seems interfering host cellular processes to ensure its intracellular persistence.

Together, these results support that resistance to *B. ostreae* in *O. edulis* is strongly linked to a large chromosomal inversion acting as a structural “supergene”. Development of a cost-effective inversion-based genotyping tool enables selective breeding and restoration strategies aimed at enhancing resilience of European flat oyster populations.

**Keywords**: *Ostrea edulis, Bonamia ostreae*, chromosomal inversions, disease resistance

# EVOLUTION OF THE TRIM FAMILY OF E3 UBIQUITIN LIGASES IN TELEOST FISH

Pérez-Pereira, N. [1], Veiga-Rúa, S. [1], Rodríguez-Vázquez, R. [1], Gallo, M. [1], González-Sánchez, A. [1], Suárez-Rivas, A. [1], Torres-Sedano, M. [1], Rodríguez-Fernández, M. [1] and Robledo, D. [1,2]

*[1]Departamento de Zoología, Genética y Antropología Física, Universidade de Santiago de Compostela, Santiago de Compostela, Spain; [2]The Roslin Institute, Edinburgh, Scotland.*

e-mail: noelia.perez.pereira@usc.es

The ubiquitin system comprises a diverse set of enzymes responsible for the post-translational modification of proteins through the ubiquitination cascade. This process can regulate the turnover, function, or cellular location of proteins, making it essential for many cellular functions. Among the components of this system, E3 ubiquitin ligases are the most diverse and regulate the final step of the cascade by transferring ubiquitin to specific target proteins. The tripartite motif (TRIM) family represents a large and highly diverse set of E3 ubiquitin ligases that has frequently been associated with antiviral responses. This family has undergone multiple expansions during vertebrate evolution, with particularly notable diversification in teleost fish. However, the evolutionary relevance and functional implications of these expansions have not been thoroughly investigated. In this study, phylogenomic and molecular evolution analyses were used to investigate the evolutionary history of three major teleost-specific TRIM expansions: the finTRIM (fish-novel TRIM), TRIM35-like, and btr (bloodthirsty-related TRIM) subfamilies. A representative set of fish species covering the main clades of the teleost phylogeny was selected from publicly available annotated genomes, in addition to selected representatives of other vertebrate clades. In addition, three clade-focused datasets including species from the orders Cichliformes, Spariformes, and Salmoniformes were analysed to study TRIM evolution in shorter evolutionary time frames. Phylogenomic orthology analyses showed that these three TRIM subfamilies are among the largest gene families across the datasets, with a clear enrichment in gene copy number in teleosts compared with other vertebrates. Changes in gene family size were statistically significant in most of the species evaluated based on computational analyses of gene family evolution. Phylogenetic inference revealed consistent clade-specific expansion patterns throughout the teleost phylogeny. Most expansions appear to originate from ancestral duplications shared among cichlids, sparids or salmonids, while putative species-specific duplications occur on a much smaller scale. Overall, the results indicate dynamic events of gene gains and losses across the teleost lineage. However, the functional consequences of these events and their potential role in immune responses remain to be clarified.

**Keywords**: TRIM family, Phylogenomics, Molecular evolution, Teleost, Gene family expansion

# PROLONGED LIGHT STRESS INFLUENCES EXPRESSION OF TRANSPOSABLE ELEMENTS IN *GADUS MORHUA*

Rudnik, L. M.[1], Silva, D. M. S.[2], Colonna, L.[3], Konstantinidis, I.[3], Vilas-Boas, L. A.[4], Paschoal, A. R.[1,5] and Fernandes, J. M. O.[2]

*[1]Federal Technological University of Paraná, Brazil, [2]Institute of Marine Sciences (ICM-CSIC), Spain, [3]Nord University, Norway, [4]State University of Londrina, Brazil, [5]Rosalind Franklin Institute, United Kingdom*

e-mail: lorenarudnik43@gmail.com

Transposable elements (TEs) are mobile DNA sequences capable of replicating and inserting into new genomic locations, representing a key driving force in evolution and genomic plasticity. Their propensity to transpose and increase copy number is rigorously attenuated by host regulatory mechanisms. Over the long term, persistently reduced activity may lead to the gradual elimination of these elements. In the present study, we investigated the impact of prolonged light stress on TE dynamics in the transcriptome of Atlantic cod (*Gadus morhua*). A total of 36 Illumina RNAseq libraries prepared from liver, were analyzed from specimens divided into two groups and exposed for six months to different photoperiods: ambient light and continuous light, each containing 9 males and 9 females. Read quality was assessed with FastQC and de novo transcriptome assembly was performed using Trinity. For the annotation and identification of transposable elements, we employed RepeatModeler2 and RepeatMasker. Our analysis revealed a significantly elevated number of transposable elements in the samples subjected to continuous light stress when compared to those kept under ambient light conditions. These results support the notion that environmental stress factors, such as photoperiod manipulation, MAY influence TE activity, thereby contributing to genomic and adaptive plasticity in Atlantic cod. These findings highlight that common aquaculture practices may induce genomic stress responses that could impact long-term robustness in cod.

**Keywords:** Transposable elements, photoperiod, TE activity

**Acknowledgements:** This study received financial support from the Research Council of Norway (EPICOD, project No. 336112), the Spanish Ministry of Science and Innovation grant PID2023-146286OB-I00 “Holosex”, grant CEX2024-001494-S funded by AEI 10.13039/501100011033), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) under the PDSE Program nº 26/2024.

# THE EFFECTS OF HEATWAVES ON ATLANTIC SALMON INFECTED WITH *YERSINIA RUCKERI*

Tengs, T.[1], Lazado, C.[1], Fernandes, J.M.O.[2,3], Siqueira, K.[4], Milton, C.I.J.[5], Macqueen, D.J.[5], Silva, D.M.[3] and Burgerhout, E.[1]

*[1]Nofima, Norway, [2]Nord University, Bodø, Norway, [3]Institute of Marine Science (ICM), Barcelona, Spain, [4]State University of Londrina, Londrina, Paraná, Brazil, [5]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom*

e-mail: torstein.tengs@nofima.no

Teleosts are known to be sensitive to changes in the environment, and an increase in the frequency of heatwaves as well as an overall increase in sea temperatures will affect aquaculture, especially when it comes to species that are reared under cold conditions. Certain developmental stages are likely to be more sensitive to changes in the environment, and the effects can be long lasting and have diverse phenotypic consequences. The molecular mechanisms underlying this plasticity are not fully understood. Hence, we have exposed Atlantic salmon (*Salmo salar*) to different temperatures during embryogenesis and subsequently transferred them to an identical environment. At the parr stage, the fish were challenged with *Yersinia ruckeri*, and head kidney samples were taken 24 hours and 72 hours post infection. RNA-Seq analyses and full genome, single cytosine resolution EM-seq analyses revealed different molecular signatures associated with temperature modulation. The different thermal regimes also seemed to have an impact on the way the fish responded to being challenged with a pathogen. Differences in both gene expression patterns and DNA methylation patterns were functionally annotated and potentially important pathways and molecular mechanisms identified.

## Posters — Nutrition & growth

# FUNCTIONAL MODULATION OF GUT BACTERIAL COMMUNITIES IN GILTHEAD SEABREAM BY DIETARY ASTAXANTHIN

Panteli, N.[1], Mastoraki, M.[2], Moulistanos, A.[1,3,4], Kaitetzidou, E.[1,3], Sidera, P.[1], Papamatthaiaki, K.[1], Chatzifotis, S.[2], Papakostas, S.[4] and Antonopoulou, E.[1]

*[1]Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Hellenic Center of Marine Research, Heraklio, Greece; [3]Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece; [4]International Hellenic University, Thessaloniki, Greece*

e-mail: amoulist@bio.auth.gr

Although aquaculture represents a cornerstone of global food production, cultured fish are frequently subjected to multiple concurrent stressors, including metabolic waste accumulation, high stocking density and handling. Astaxanthin, a lipid-soluble keto-carotenoid naturally produced by microorganisms and plants, is widely used as a feed additive to impart coloration. Beyond pigmentation, astaxanthin exhibits antioxidant, immunomodulatory, and growth-promoting properties, and simultaneously modifies gut microbiota. Considering that diet is a key microbiota determinant, the present study aims to assess the gut bacterial dynamics of gilthead seabream, *Sparus aurata*, following dietary supplementation of astaxanthin. To address this, four diets containing increasing levels of whole microalga *Haematococcus pluvialis* powder were formulated to provide 0, 20, 50 and 100 mg/kg astaxanthin to gilthead seabream in a 107-day feeding trial. The gut-associated bacterial communities were characterized via 16S rRNA gene amplicon sequencing on a MinION platform. Higher astaxanthin inclusion shifted both the structure and the composition of the gut microbiota, while promoting the formation of ecological niches that prompted the colonization of unique bacterial taxa. Furthermore, dietary astaxanthin affected the bacterial interactions, with alterations in the communities' functional potential. Combined with growth and physiological outcomes, these results highlight the potential of dietary astaxanthin to modulate gut microbiota and support fish health under intensive aquaculture conditions. This study provides a comprehensive framework for integrating functional microbiota data into nutritional strategies in aquaculture.

**Keywords**: Gut Microbiota, Metagenomics, 16S rRNA, Astaxanthin, Teleost

# ENDOCRINE GENE EXPRESSION ASSOCIATED WITH GROWTH AND LIPID DEPOSITION UNDER ALTERNATIVE DIETS IN GILTHEAD SEABREAM

Oikonomou, S.[1,2], Angelakopoulos, R.[3], Tekeoglou, M.[1], Tsipourlianos, A.[3], Kazlari, Z.[1], Loukovitis, D.[4], Dimitroglou, A.[5], Giannoulis, T.[3], Mamuris, Z.[3], Chatziplis D.[1] and Moutou, K.[3]

*[1]International Hellenic University, Greece; [2]Research Institute of Animal Science, ELGO Demeter, Greece; [3]University of Thessaly, Greece; [4]University of Patras, Greece; [5]University of Athens, Greece*

e-mail: valiaekonomou@hotmail.com,
e-mail: rangelak@uth.gr

The increasing replacement of fish meal and fish oil with plant-based ingredients is a major transition in modern aquaculture feeds. However, the physiological and genetic mechanisms underlying fish responses to these dietary shifts remain poorly understood. In gilthead seabream (*Sparus aurata*), metabolic regulation of growth and lipid deposition is strongly influenced by endocrine pathways involving growth hormone signalling and nutrient transport. In the present study, we investigated the effects of a dietary shift from a traditional fish meal/oil diet (FM) to a partially plant-based diet (PP) (details in formulation, see in Oikonomou et al., 2023) on growth performance, muscle fat content, and hepatic expression of key metabolic genes. A total of 160 fish were evaluated, with final body weight (BW) and muscle fat (FAT) recorded at 549 DPH. Hepatic expression levels of *ghri, igf1*, and *ttr* were quantified and incorporated as predictors in mixed animal models accounting for additive genetic effect. Diet significantly affected both BW and FAT. Fish fed the FM diet showed higher BW, whereas individuals fed the PP diet displayed increased FAT. Gene expression analyses revealed that hepatic *ttr* and *igf1* expression were negatively associated with BW, while *ghri* expression showed a positive association with FAT. These findings suggest that endocrine signalling pathways involved in growth hormone regulation and metabolic transport contribute to the physiological response to plant-based diets. By incorporating pedigree-based additive genetic effect, the analysis also highlights that variation in growth and lipid deposition is partly linked to genetically influenced metabolic pathways, suggesting potential targets for nutrigenomic-informed selective breeding in gilthead seabream. Our findings highlight the potential role of gene expression markers in understanding metabolic adaptation to alternative feeds and provide insights relevant for nutritional strategies and selective breeding programs in seabream aquaculture.

**Keywords**: gene expression, plant-based diet, gilthead seabream

***Reference***

Oikonomou, S.; Kazlari, Z.; Loukovitis, D.; Dimitroglou, A.; et al., 2023. Genetic Parameters and Genotype × Diet Interaction for Body Weight Performance and Fat in Gilthead Seabream. Animals 13, 180.

# HALOPHYTE EXTRACTS TEMPER THE FUNCTIONS OF ATLANTIC SALMON HEAD KIDNEY ADHERENT LEUKOCYTES AND GILL EPITHELIAL CELLS

Ferreira, M.[1]*, Stein, J.K. [2,3], Fredsgaard M.[2], Rudnyckyj S.[2], Solhaug A.[4], Gjessing M.[4], Thomsen M. H.[2] and Viswanath, K.[1]

*[1]Nord University, Bodø, Norway; [2]Aalborg University, Esbjerg, Denmark; [3]Halorefine APS, Denmark; [4]Norwegian Veterinary Institute, Ås, Norway*

*e-mail:* kiron.viswanath@nord.no

Recent advancements in aquaculture have prioritized the development of functional feed additives to enhance fish health. Specifically, there is increasing interest in screening plant-derived compounds for immune-enhancing properties, including those from the halophyte, *Salicornia ramosissima*. In this study, we assessed the impacts of various *S. ramosissima* extracts on Atlantic salmon cells, utilizing both functional assays and transcriptomic analyses to evaluate health-protective characteristics. Adherent leukocytes (primary cells) from head kidney and a gill epithelial cell line (ASG-10) of Atlantic salmon were exposed to different types of *S. ramosissima* extracts (codes: EXT3, XAD and FERM), obtained from distinct steps of the extraction cascade. Cells were exposed to suitable concentrations of each extract for either 3 or 24h, followed by a challenge with inactivated pathogenic bacteria *Tenacibaculum maritimum*. Phagocytosis and reactive oxygen species (ROS) production were quantified by employing imaging flow cytometry protocols. Furthermore, a wound healing assay was performed on gill epithelial cells using an automated scratch system followed by live cell imaging. In parallel, a snapshot of the changes in the transcriptome of the two cell types was captured to reveal their molecular responses. The halophyte extracts induced cell-specific responses, indicating its potential as functional aquafeed additives for Atlantic salmon. Adherent leukocytes exhibited significant immune activation (enhanced phagocytosis and ROS production) after 24h exposure to XAD, driven by earlier transcriptional reprogramming and molecular priming observed at 3h. In epithelial cells, XAD increased ROS production at 24h without altering phagocytosis, suggesting stress adaptation via metabolic pathway modulation (e.g., glutathione metabolism, xenobiotic response and iron ion homeostasis). Conversely, EXT3 and FERM accelerated wound healing, with EXT3 specifically affecting cell migration and tissue remodeling pathways. These findings suggest that XAD enhances immune activation while EXT3/FERM improves tissue integrity, making these extracts capable of supporting salmon gill barrier integrity.

**Keywords**: Plant extracts, gill cell line, head kidney leukocytes, tissue integrity, transcriptome.

Acknowledgments: This study was co-funded by the European Union (Project: IGNITION - GA 101084651) and the UK Research and Innovation (UKRI). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union, the Research Executive Agency (REA) or the UKRI. Neither the European Union nor the granting authorities can be held responsible for them.

# *LACTOCOCCUS LACTIS* AND (1,3)-β-GLUCAN DRIVE REMODELING OF IMMUNE RESPONSES AND MICROBIAL METABOLIC PROFILE IN ZEBRAFISH

Kato, H.[1], Chowdhury, S.[1], Ferreira, M.[1], te Walvaart, M.M.M.[1], Utasa D.N.[1], Ahmed, N.A.[1], Bisa, S.[1], Latif, Md.A.[1], Sørensen M.[1], and Viswanath, K.[1]

*Nord University, Bodø, Norway*

*e-mail:* kiron.viswanath@nord.no

Functional feeds are crucial for a resilient and sustainable aquaculture industry. The bioactive components in these feeds must be recognized by host factors or utilized by gut microbes to activate signaling pathways, eliciting specific immune responses and drive the production of host-relevant metabolites, thereby realizing their full functional potential. We investigated the probiotic and synbiotic effects of *L. lactis*, alone and in combination with (1,3)-β-glucan (synbiotic diet) derived from the microalga, *Euglena gracilis* on zebrafish. The probiotic diet primarily enriched GO terms related to extracellular matrix organization/assembly, elastic fiber assembly, cellular component assembly and biogenesis among the upregulated differentially expressed genes (DEGs). In contrast, downregulated DEGs were enriched in GO terms associated with ion transport and mRNA/RNA catabolic process, regulation/negative regulation of mRNA/RNA metabolic processes, and nucleosomal/chromatin binding. Additionally, upregulated DEGs displayed enrichment in GO terms related to RNA polymerase-associated pathways, GTPase activity, G protein activity, and cyclin-dependent protein kinase regulatory activity. Furthermore, enriched KEGG pathways associated with altered metabolites reflected broad shifts in amino acid metabolism, neurotransmission-related pathways (including serotonergic synapse), and redox homeostasis. KEGG enrichment further suggested microbial contributions to metabolome. The synbiotic diet primarily enriched GO terms related to positive T cell selection, chemokine/cytokine mediated signaling pathway, response to chemokine/ cytokine, Tcell / mononuclear cell / lymphocyte / leukocyte differentiation /activation, lysosome/lytic vacuole organization, among the upregulated differentially expressed genes (DEGs). In contrast, downregulated DEGs were enriched in GO terms associated with several pathways related to transport/transporter activity, channel activities and phosphorylation. The enriched KEGG pathways associated with the altered metabolites reflected several metabolic features that were shared with the probiotic diet-fed group. Interestingly, dopaminergic synapse along with serotonergic synapse was also enriched among the neurotransmission-related pathways, and microbial contributions to the metabolome was also evident in the synbiotic group. The distinct transcriptomic and metabolomic shifts observed in the probiotic and synbiotic groups highlight the specific actions of *L. lactis*, with certain responses further amplified in the presence of (1,3)-β-glucan.

**Keywords**: Probiotic-host interaction, synbiotic, intestinal transcriptome, intestinal metabolome

## Posters — Microbial genomics

# NOVEL QPCR ASSAY OPTIMISED FOR ANALYSIS OF PHAGE THERAPY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND RAMIFICATIONS FOR GUT MICROBIOME

James, L. [1], Overland, B.[1], Shankregowda, A.M.[1], Uren Webster, T.M.[1], Thomas, G. [1] and Consuegra, S.[1,2]

[1]*Department of Biosciences, Centre for Sustainable Aquatic Research, Swansea University, Swansea, UK, SA2 8PP,*
[2]*Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain.*

email: 2131640@swansea.ac.uk

Given the impact of *Flavobacterium psychrophilum* on the health and yield of salmonid aquaculture worldwide, a growing field of research has considered its mitigation through bacteriophage therapy, with flavophage strain FpV4 recognised amongst the most promising candidates for therapeutic application in aquaculture. Whilst the antibacterial potential of flavophage FpV4 in controlling *F. psychrophilum* outbreaks has been demonstrated empirically, there remain significant knowledge gaps regarding the effects of FpV4 phage administration upon the host microbiome. Quantitative PCR (qPCR) has been shown to provide accurate and highly sensitive enumerations of phage copies for analysis of phage therapies across numerous works, but no published qPCR protocol is currently available for the detection and quantification of phage FpV4. We have developed and optimised a novel qPCR assay for FpV4 phage and demonstrated its utility for application on biological samples derived from FpV4 phage-fed rainbow trout (*Oncorhynchus mykiss*). Our results elucidate the retention and persistence of the administered phage in treated fish to inform further optimisation of therapeutic regimens. Further, our assay provides preliminary insight into the influence of phage treatment upon the gut microbiome of rainbow trout and its potential effects upon the rate of structural change and diversification of microbial communities.

**Keywords**: *Flavobacterium psychrophilum*, *Oncorhynchus mykiss*, phage therapy, FpV4, qPCR.

# *IN SILICO* CHARACTERIZATION OF TYPE VI SECRETION SYSTEMS IN EM-90 AND LF-89 GENOGROUPS OF *PISCIRICKETTSIA SALMONIS*

Sandoval, A.[1,2], Valenzuela-Miranda, D.[1,2] and Gallardo-Escárate, C.[1,2]

[1]*Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, P. O. Box 160-C, Concepción, Chile.* [2]*Laboratory of Biotechnology and Aquatic Genomics, Department of Oceanography, University of Concepción, Concepción, Chile.*

e-mail: asandovalconcha1998@gmail.com

*Piscirickettsia salmonis* is the main bacterial pathogen affecting Chilean salmon farming, causing Salmonid Rickettsial Septicaemia and severe economic losses worldwide. Although its Dot/Icm type IVB secretion system has been extensively studied, the Type VI secretion system (T6SS) of this intracellular pathogen remains poorly characterized at the genomic level. This study aimed to perform an in-silico characterization of T6SS architecture, diversity and putative immunity systems across 72 high-quality genomes representing the EM-90 and LF-89 genogroups of *P. salmonis*, using the most representative sequences of each genogroup for phylogenetic analyses. Genotyping was conducted using Bayesian phylogeny (MrBayes, 20,000,000 generations, 10% burn-in, TRN+G model) with *C. burnetii* and *F. tularensis* as outgroups. A custom Python/Conda pipeline was implemented to screen all proteomes with T6SS HMM profiles and to validate candidate hits against the SecReT6 database, enabling identification and genomic localization of T6SS gene clusters. Systems were classified using the contractile sheath component TssB and its non-canonical Francisella ortholog IglA to infer Bayesian phylogenies. All genomes harboured T6SS genes, and TssB-based phylogenies robustly placed *P. salmonis* systems within subtype I1 (*Myxococcus xanthus*-like). Notably, EM-90 isolates encoded two distinct T6SS modules: a subtype I1 system and a subtype II system related to *F. tularensis.* None of the genomes carried a complete set of 13 canonical T6SS core genes; instead, the systems correspond to reduced T6SS architectures. SecReT6 analyses detected on average six genes for subtype I1 (TssL, TssK, TssJ, TssB, TssC and TssE) and four genes for subtype II (two copies of IglA and two copies of IglB, orthologs of TssB/TssC). In contrast, our HMM-based pipeline recovered eight I1-related genes (ClpV, PAAR, TssB, TssH, TssK, TssI, TssL and VgrG) and four II-related genes (two IgIB variants plus single IgIC and PdpA) with more than 95% query coverage, revealing a more extensive T6SS repertoire than that reported by SecReT6. In silico “predator–prey” immunity assays between EM-90 and LF-89 indicated a low-risk scenario compatible with effective sister-immunity, suggesting that these genogroups may coexist within the same host cell without mutual T6SS-mediated killing, thereby facilitating coinfection and more severe disease. Overall, EM-90 and LF-89 carry reduced yet functionally diverse T6SS repertoires, including a dual subtype I1/II architecture in EM-90 that may underlie its higher virulence, and non-canonical IglA/IglB modules emerge as promising targets for future functional studies, vaccine development and novel control strategies against piscirickettsiosis.

**Keywords**: *Piscirickettsia salmonis*, T6SS, Bacterial Virulence, Salmon Aquaculture

# ADVANCED PCR-BASED TOOLS FOR EARLY PATHOGEN IDENTIFICATION AND DISEASE MANAGEMENT IN AQUACULTURE

Freitas, I.[1*], Silva, I.A.L. [1*], Angelo, M.[1], Sousa, R. [1], Livramento, M. [1], Pousão-Ferreira, P. [1,2] and Lourenço-Marques, C.[1,2]

*[1] S2AQUA, Olhão, Portugal, [2] IPMA/EPPO, Olhão, Portugal; *Contributed equally.*

e-mail: iris.silva@s2aquacolab.pt

The intensification of aquaculture systems has increased the risk of disease outbreaks, reinforcing the need for rapid, sensitive, and reliable diagnostic tools to detect pathogens affecting fish and bivalves. In this context, molecular methodologies have emerged as transformative approaches, enabling early and accurate identification of infectious agents, often prior to the onset of visible clinical symptoms. At S2AQUA, a comprehensive suite of molecular diagnostic protocols has been developed and optimized, primarily based on polymerase chain reaction (PCR) and quantitative PCR (qPCR), to support pathogen surveillance and health management in aquaculture. These tools rely on the design of highly specific primers derived from publicly available genomic databases, targeting conserved regions that allow precise discrimination among closely related taxa. The implemented assays cover a broad spectrum of marine and freshwater parasites, including *Amoebae*, *Amyloodinium ocellatum*, *Microsporidia*, *Myxozoa*, *Monogenea*, *Tetracapsuloides bryosalmonae*, and *Kudoa* species. In parallel, protocols have been established for the detection of key bacterial pathogens impacting Mediterranean aquaculture, such as Photobacterium damselae subsp. damselae and piscicida, Tenacibaculum maritimum, soleae and discolor, Aeromonas salmonicida and hydrophila, Pseudomonas aeruginosa and anguillispetica, and multiple Vibrio species associated with fish, shrimp, and bivalve production. To enhance efficiency, multiplex PCR assays were designed, allowing simultaneous detection of multiple pathogens within a single reaction, thereby reducing time, cost, and sample processing requirements. Additionally, standardized protocols have been implemented for the detection and quantification of viral agents, including Nodavirus in fish and Norovirus in bivalves, following internationally recognized guidelines. Collectively, these molecular approaches provide robust, high-throughput diagnostic capabilities that strengthen disease monitoring, improve response times, and support the development of more resilient and sustainable aquaculture systems through genomics-informed health management strategies.

**Keywords**: Molecular diagnosis, aquaculture, bacteria, parasites, virus, qPCR

**Acknowledgments:** This study had the support of the projects WP9- Portuguese Blue Biobank under the Blue Economy Pact - Project Nº. C644915664-00000026, Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148) and SAUDE&AQUA II (MAR-021.1.3-FEAMPA-00018).

# EXPLORING MUSSEL MICROBIOMES IN THE KRKA RIVER ESTUARY

Trumbić, Ž.[1], Ćurko, T.[1], Hrabar, J.[2], Bogdanov, L.[1] and Petrić, M.[1]

[1] *University of Split, Faculty of marine sciences, Croatia,* [2] *Institute of Oceanography and Fisheries Split, Croatia*

e-mail: ztrumbic@unist.hr

*Mytilus galloprovincialis*, commonly referred to as the Mediterranean mussel, is an important shellfish species in Croatian marine aquaculture, with an average production of 926 tonnes (live weight) recorded over 2021–2023. Increasing research interest has focused on the mussel microbiome due to its essential role in mussel physiology, health, and aquaculture performance. Beyond their value as a food source, mussels function as natural biofilters, removing suspended particles and potentially harmful microorganisms from the water, thereby contributing to improved water quality and overall ecosystem health. This study examined the complete microbial community present in the tissues (gills and hepatopancreas) of farmed mussels, as well as in seawater from the Krka River estuary, with monthly sampling conducted throughout 2022 and 2023 and by employing DNA metabarcoding of the V4–V5 region of the 16S rRNA gene. Analysis showed that Proteobacteria, Bacteroidota, and Firmicutes were the dominant bacterial phyla in mussel tissues, displaying clear seasonal fluctuations. A total of 648 bacterial genera were identified, of which 522 were detected in gills, 516 in the hepatopancreas, and 498 in seawater samples; 331 genera were shared across all three sample types. Potentially pathogenic genera and coliform bacteria, including Vibrio, Clostridium, and the Escherichia–Shigella group, were present at very low abundances and were detected exclusively in the hepatopancreas. Alpha diversity was highest in seawater and lowest in both gill and hepatopancreas samples. Overall, these findings improve understanding of host–microbe–environment interactions in mussels and provide valuable insights for optimizing aquaculture practices and promoting sustainable mussel farming.

**Keywords:** *Mytilus galloprovincialis*, microbiome, hepatopancreas, gills, Krka River estuary, aquaculture

## Posters — Integrative omics

# EXPLORING INTERGENERATIONAL EPIGENETIC EFFECTS IN ATLANTIC SALMON AQUACULTURE: THE EPIBROOD PROJECT

Appel, R.J.C. [1], Saito, T. [2], Fjelldal, P.G. [2], Folkedal, O. [2], Adam, A.-C. [2], Robinson, N.A. [3], Fernandes, J.M.O. [1], Piferrer, F. [1] and Skjærven, K.H. [2]

*[1]Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain; [2]Institute of Marine Research (IMR), Bergen, Norway; [3]The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), Tromsø, Norway.*

e-mail: rjcasarotto@icm.csic.es

Intergenerational epigenetic effects are increasingly recognized as an important mechanism through which environmental conditions experienced by broodstock can influence offspring phenotypes. The EpiBrood project investigates whether broodstock rearing environments and maturation practices used in Norwegian salmon aquaculture can induce epigenetic changes that affect phenotypic traits, such as growth, in subsequent generations of production fish. The project integrates industry practices with multi-omics analyses to explore potential parent-offspring epigenetic effects in Atlantic salmon. At the Matre research station (Norway), broodstock were exposed to a controlled light treatment between 2023 and 2024, followed by repeated delousing procedures between 2024 and 2025 involving short-term thermal stress (32 °C for 30 s) applied every second month prior to transfer to freshwater approximately six months before spawning. Biological samples from broodstock (F0) and their offspring (F1) were collected to assess potential phenotypic effects across generations. Preliminary observations suggest that these treatments may influence certain traits in the parental generation, with initial indications of potential effects on fecundity and egg mortality in the offspring. To investigate the molecular mechanisms underlying these effects, genome-wide DNA methylation profiles will be analyzed using enzymatic methyl-sequencing (EM-seq), enabling the investigation of methylation patterns across genic and genomic regions. Initial analytical steps include the evaluation of beta value distributions across genomic features and gene expression groups. In parallel, transcriptomic analyses will be conducted to explore gene expression dynamics and regulatory RNA molecules, including the identification of transcript isoforms and long non-coding RNAs using a two-pass STAR and StringTie pipeline. In addition, alternative bioinformatic workflows for EM-seq analysis are being evaluated to improve robustness in non-model organisms. Comparative assessments of different tools for read processing, alignment, and differential methylation detection are being performed. Together, these approaches aim to establish a framework for understanding epigenetic regulation in salmon broodstock and its potential implications for aquaculture production and management practices.

**Keywords**: Epigenetics, DNA methylation, Intergenerational effects, Atlantic salmon, Aquaculture, Multi-omics

# CRUDE OIL-INDUCED EPIGENETIC ALTERATIONS IN THE POLAR COD (*BOREOGADUS SAIDA*) REPRODUCTIVE AXIS: INSIGHTS FROM EM-SEQ

Chapman, A.L.[1], Fernandes, J.M.O.[2], Silva, D.M.[2], Erhart, C.[1], and Nahrgang J.[1]

[1]*The Arctic University of Norway (UiT), Tromsø, Norway,* [2] *Institut de Ciències del Mar (ICM), Barcelona, Spain*

email: Abigail.chapman@uit.no

The Arctic ecosystem faces increasing threats from oil pollution due to expanding industrial activities. Exposure to petroleum-derived compounds has been shown to induce intragenerational epigenetic modifications in teleost fish, particularly affecting the brain-pituitary-gonad-liver (BPGL) axis through alterations in DNA methylation patterns. These epigenetic modifications can lead to dysregulation of key endocrine pathways, potentially impacting gametogenesis, reproductive behavior, and overall population dynamics in affected aquatic ecosystems. Despite evidence of crude oil-induced reproductive disruption in some Arctic species, the absence of mechanistic epigenetic data hampers our ability to predict how such stressors affect the resilience and reproductive timing these populations. The present study investigates the impacts of a Goliat crude oil water soluble fraction (WSF) on epigenetic markers in adult polar cod (*Boreogadus saida*), a key species in Arctic food webs. During an 89-day experiment, adult polar cod in late vitellogenesis were exposed for 20 days to two concentrations of crude oil WSF, and monitored alongside unexposed controls until spawning. Oil exposure induced a shift in the gonadal maturation window, without histological alterations of the gonads but a dose-dependent advancement of spawning, accompanied by alterations in plasma sex steroids and modulation of ovarian steroidogenesis genes. We hypothesize that exposure to crude oil induces tissue-specific changes in DNA methylation along the reproductive axis, which disrupts the regulation of endocrine pathways and thus contributes to altered spawning patterns. Gonad and liver tissues (n=30 each, balanced by sex) were analyzed by enzymatic methylation sequencing, followed by stratified pairwise analysis in R, to detect exposure-related changes in DNA methylation patterns across genes involved in the reproductive axis and investigate the changes in spawning phenology. These findings provide key insights into the molecular mechanisms underlying endocrine disruption and altered spawning phenology, highlighting potential consequences for reproductive success and population dynamics in Arctic fish exposed to petroleum-derived compounds, and are directly relevant for coastal aquaculture where similar contaminant-driven epigenetic effects could impact broodstock performance and spawning time.

**Keywords:** EM seq, Ecotoxicology, Crude oil, Fisheries, Epigenetics

# OYSTER: EMPOWERING EARLY CAREER RESEARCHERS IN MARINCE SCIENCE ACROSS EUROPE

Chapman, A.L.[1]

[1]*on behalf of OYSTER (Orienting Young ScienTists of EuromaRine)*

email: oyster@euromarinenetwork.eu

Early career researchers (ECRs) play a critical role in advancing marine science, yet they often face challenges related to funding access, networking opportunities, career development, and visibility within the scientific community. OYSTER (Orienting Young Scientists of Euromarine) is the ECR branch of Euromarine, the largest European network dedicated to marine research. OYSTER was founded in 2018 to address these challenges by fostering a supportive, inclusive, and resource-rich environment for emerging marine scientists across Europe. This poster presents the structure, initiatives, and impact of OYSTER in supporting ECRs at multiple stages of their careers. Key activities include the evaluation and distribution of funding opportunities for courses, conference participation, internships, collaborative research projects, and individual fellowships. OYSTER also facilitates professional development through a mentorship program, the organization of ECR-focused events at international conferences, and a podcast series that highlights both the scientific work and personal journeys of early career researchers. In addition, OYSTER actively promotes visibility and community engagement through targeted social media campaigns that disseminate job opportunities, training resources, and institutional activities within the Euromarine network. Collaborative efforts with other research networks further expand opportunities for interdisciplinary exchange and professional growth. By providing accessible resources, amplifying ECR voices, and strengthening connections across the European marine science community, OYSTER contributes to a more equitable and dynamic research landscape. This poster aims to raise awareness of these opportunities and encourage broader participation from ECRs seeking to enhance their careers and scientific impact.

**Keywords:** Marine science, Early career researchers, Outreach

# GENOME-WIDE IDENTIFICATION OF LOCI AND IMMUNE-RELATED CANDIDATE GENES FOR RESISTANCE TO SALMON RICKETTSIAL SYNDROME IN ATLANTIC SALMON

Gonzalez-Prendes, R.[1], Bickhart, D. M. [1] and Jansen K.[1]

[1] *Hendrix Genetics B.V., 5831 CK Boxmeer, The Netherlands*

e-mail: Rayner.Prendes@hendrix-genetics.com

Salmon rickettsial syndrome (SRS) is a major disease in Atlantic salmon farming and causes substantial economic losses. In this study, we analysed binary mortality records from 8,052 Atlantic salmon together with 7,353 single nucleotide polymorphisms (SNPs) to identify genomic regions associated with SRS resistance. Significance was evaluated using false discovery rate (FDR) correction at two levels: across all markers (genome-wide) and within each chromosome (chromosome-level), retaining loci with $q < 0.05$. Genome-wide significant SNPs were detected on *Salmo salar* chromosomes (SSA) 2, SSA4, SSA9, SSA10, and SSA19. The strongest significance were located on SSA2 at 54.77 Mb and SSA10 at 81.73 Mb. At chromosome level, additional significant SNPs were identified on SSA2, SSA8, and SSA10. Key positional candidate genes included *evpla* (envoplakin a) on SSA2, *dnajc18* on SSA4, *banp* on SSA10, and *epb41l3a* on SSA19, involved in cytoskeletal organization, protein homeostasis, and transcriptional regulation. In addition, pathway analysis of genes within a 500 kb window around the top SNPs highlighted innate immune signaling (Toll-like receptor, NF-κB, and interferon pathways). Overall, our results indicate that variation in SRS mortality is influenced by multiple loci. In addition, we have two direct applications for improving SRS resistance in salmon. At a breeding level, the identified significant SNPs can be used in prediction models by weighting their effects to improve genomic selection. At the molecular level, the candidate genes provide direct targets for functional validation and future gene-editing studies.

**Keywords:** Atlantic salmon; salmon rickettsial syndrome; genomewide association study; disease resistance; SNP; genomic selection; gene editing

# TRANSCRIPTOMIC INSIGHTS INTO ATLANTIC SALMON IMMUNE RESPONSES TO *PISCIRICKETTSIA SALMONIS* GENOGROUPS

Leal, Y [1], Casuso, A.[1], Valenzuela-Muñoz, V.[1] and Gallardo-Escárate, C.[1]

[1]*Interdisciplinary Center for Aquaculture Research, University of Concepción, Concepción, Chile*

e-mail: yleal@udec.cl

Salmonid Rickettsial Septicemia (SRS), caused by the intracellular bacterium *Piscirickettsia salmonis*, is one of the most significant infectious diseases affecting Atlantic salmon aquaculture in Chile. Two major genogroups, LF-89 and EM-90, have been described, yet their effects on the host immune response remain incompletely understood. In this study, we investigate the transcriptomic responses of Atlantic salmon head kidney during infection with *P. salmonis* genogroups. Fish were distributed into four experimental groups: control, infection with EM-90, infection with LF-89, and co-infection with both genogroups. Preliminary transcriptomic analyses revealed distinct immune expression patterns between genogroups. Infection with EM-90 showed increased expression of B-cell marker genes, including *ebf1*, *vav2.1*, *blnk*, and *IgM*, whereas T-cell markers such as *cd3e*, *cd28*, and *skap1.2* were downregulated, suggesting modulation of T-cell-associated responses. Together, these findings highlight genogroup-specific transcriptional responses in Atlantic salmon immune tissues and provide a framework to identify molecular markers associated with infection dynamics. This work contributes to a better understanding of host–pathogen interactions and may support the development of improved strategies for SRS control in salmon aquaculture.

**Keywords:** Atlantic salmon, *Piscirickettsia salmonis*, transcriptomics, immune response, SRS

# A MAJOR LOCUS ON CHROMOSOME 14 IMPACTS DEVELOPMENTAL VARIATION OF ATLANTIC SALMON SMOLTIFICATION

Saitou, M. [1], Manousi, D. [1,2], van Dalum, J. [4], Striberny, A. [3], Grønvold, L. [1], Brekke, C. [1], Boison, S.A. [6], Kwak, J. [1], Vera Ponce de León, A. [1], Gjerde, B. [5], Jørgensen, E. [4], Hazlerigg, D. [4] and Sandve, S.R. [1]

*[1]Norwegian University of Life Sciences, Ås, Norway, [2]Roslin Institute, Edinburgh, United Kingdom, [3]Nofima AS, Tromsø, Norway, [4]Arctic University of Norway, Tromsø, Norway, [5]Nofima AS Ås, Norway, [6]MOWI Genetics AS, Bergen, Norway.*

e-mail: dmanousi@ed.ac.uk

Atlantic salmon smoltification, the transition from fresh to seawater, is a complex developmental process characterized by extensive physiological and behavioral changes. Smolt development is influenced by both genetic components as well as environmental queues including photoperiod changes. Central to this transformation is the functional remodeling of the gill, a key osmoregulatory organ. However, our understanding of the genetic basis of smolt development in regards to gill physiology remains limited.

To identify the genetic architecture of smolt seawater tolerance, 2086 Atlantic salmon juveniles were exposed to three photoperiod regimes and RNA-seq data were generated from small gill biopsies. Individual genotypes were imputed from a 66K SNP array to high density (400K) and body weight was recorded for each fish. Association analysis identified 45,543 significant associations (eQTL) involving SNP and 16,314 genes expressed in gill tissue, 65% of which were shared across light regimes. Dissection of eQTL based on SNP – gene distance (cis: <500kb; trans: >500 kb) revealed a major trans-eQTL hotspot on chromosome 14 harboring over 30% of all trans- associations. Within the hotspot, a single hub-SNP controlled expression variation of 2,075 genes, analysis of which using single-cell transcriptomics approaches showed significant enrichment in 16 gill cell types known to undergo changes during smolt development. Furthermore, association analysis using body weight data detected significant signals between growth and hub-SNP genotypes whereas assessment of linkage disequilibrium (LD) surrounding the hub-SNP locus detected elevated LD overlapping the genes *pak1ip* and *endo-u*. Although variant effect prediction did not identify clear causal variants, integration of long-read sequencing data from individuals with opposite homozygous hub-SNP genotypes detected two sequence deletions overlapping exonic regions in both genes.

Collectively, the present study reveals a major regulatory locus influencing gene expression and growth, providing insights into future investigation of cell-type-specific mechanisms underlying smolt development.

**Keywords:** Atlantic salmon, smoltification, eQTL

# EPIGENETIC AND TRANSCRIPTOMIC RESILIENCE OF FISH EMBRYOS FOR FUTURE SPACE AQUACULTURE

Mehta, T. [1], Ribas, L. [2], Przybyla, C. [3], Hernández, R. [4], Peña, C. [4], Mistsopoulou, M. [2] and Joly, S. [2]

*[1]Institute of Systems, Molecular and Integrative Biology, University of Liverpool, UK; [2]Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain; [3]French Institute for Ocean Science (IFREMER), Palavas, France; [4]Laboratorio Subterráneo de Canfranc (LSC), Canfranc-Estación, Spain.*

e-mail: Tarang.Mehta@liverpool.ac.uk

Fish embryos in space-like environments must integrate multiple stressors, yet our pilot work suggests their molecular response is constrained. Building on findings from zebrafish (*Danio rerio*) and European sea bass (*Dicentrarchus labrax*) reared under simulated microgravity and altered background radiation, we present an integrative analysis of embryonic transcriptomic and epigenomic plasticity relevant to space aquaculture. At the Canfranc Surface and Underground Laboratories (LSC), we decoupled microgravityinduced and background radiation stresses—using a clinostat and a lowradiation 'cosmic silence' biolab—to generate matched RNAseq and wholegenome enzymatic methylation datasets across embryo cohorts. Initial analyses show that only a small fraction of expressed genes respond at the transcript level (≤0.4% of genes differentially expressed when contrasting microgravity *versus* control under cosmic silence), with zebrafish genes enriched for DNA damage response, replication checkpoint, mTOR signalling and tissue repair, and E. sea bass embryos under "silence + microgravity" showing activation of acutephase and innate immune pathways, pointing to finetuning of inflammatory and complement cascades as key axes of resilience. By integrating wholegenome methylation profiles with expression, we identify candidate epigenetically regulated stressresponse modules under microgravity and radiation; notably, zebrafish embryos display remarkably few differentially methylated regions (38 hyper- and 38 hypomethylated) under simulated microgravity and cosmic silence, suggesting distinct regulatory routes for gravity and radiation effects. Resolving how gene expression and DNA methylation jointly buffer, or encode, early developmental exposure to these stressors will help pinpoint molecular targets for selecting fish strains capable of thriving in closedloop aquaculture systems on space missions. e.g., lunar, with wider applications to increasingly unpredictable aquatic environments on Earth.

**Keywords:** transcriptome, methylome, space, early development

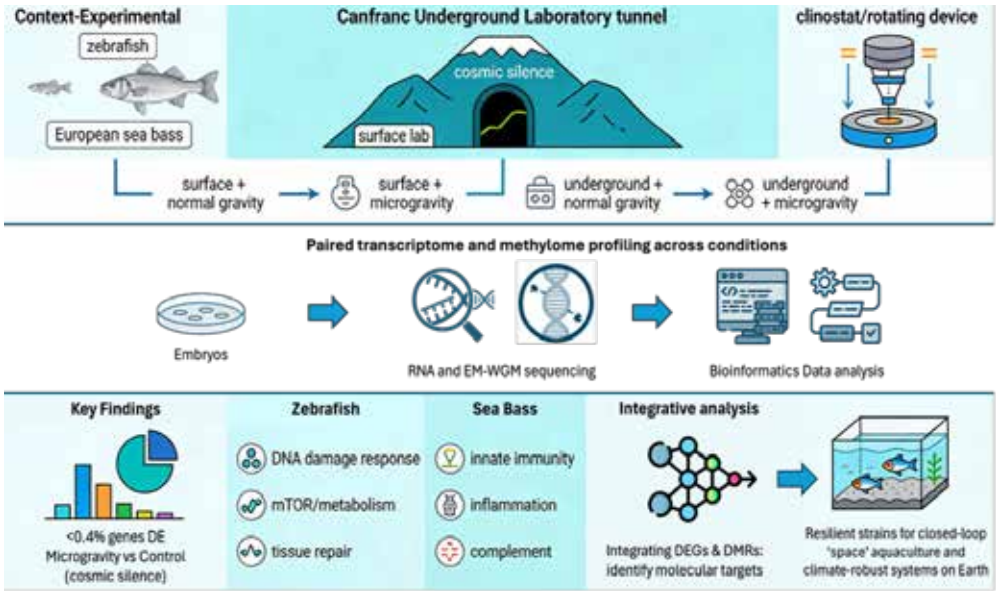



***Figure 1.*** *Schematic summarising experimental design, omics workflow and key pathways underpinning zebrafish and sea bass embryo resilience to simulated microgravity and altered radiation*

# INTEGRATING GENOMIC AND TRANSCRIPTOMIC ANALYSES REVEALS A KEY REGULATORY ROLE OF *VGLL3* IN GILTHEAD SEABREAM DOMESTICATION

Moulistanos, A.[1,2,3], Kaitetzidou, E.[1,2], Minoudi, S.[1,2], Gkagkavouzis, K.[1,2], Karaiskou, N.[1,2], Antonopoulou, E.[1], Triantafyllidis, A.[1,2] and Papakostas, S.[3]

[1]*Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece,* [3]*International Hellenic University, Thessaloniki, Greece*

e-mail: amoulist@bio.auth.gr

Selective breeding in aquaculture targets traits such as growth, physiological performance, and reproductive timing. Understanding the genomic regulators underlying these traits is critical for improving breeding strategies. The gene *vgll3* (vestigial-like family member 3) has been identified as a major regulator of life-history traits in Atlantic salmon (*Salmo salar*), accounting for a substantial proportion of the variation in age at maturation and body condition. Its conserved regulatory role across vertebrates suggests that *vgll3* may also influence key production traits in other aquaculture species. Here, we investigated the potential role of *vgll3* in the domestication process of gilthead seabream (*Sparus aurata*) using an integrative DNA- and RNA-level approach. A total of 91 individuals from nine farmed and eleven wild populations in Greece were analysed using Sanger sequencing of the *vgll3* coding region. A polymorphic site ($SNP_{vgll3}$; genotypes AA, AG, GG) showed significant differences in genotype frequencies between farmed and wild populations (Wilcoxon test, $p = 5.60 \times 10^{-3}$), with the GG genotype enriched in farmed stocks. Gene expression analyses in juveniles at 69 days post-hatching (dph), a critical developmental stage for muscle growth and physiological development in gilthead seabream, revealed significantly lower *vgll3* expression in the GG genotype compared with the AA genotype (qPCR, p = 0.027). RNA-Seq analysis further revealed consistent $SNP_{vgll3}$ genotype-associated transcriptional differences affecting key genes involved in muscle development and reproductive pathways, including *amh*, *cacng1b*, and *igfn1.1*. Together, these results support a regulatory role of *vgll3* in developmental and physiological processes relevant to aquaculture performance, highlighting it as a promising candidate gene underlying domestication and a potential target for future genomic breeding strategies in gilthead seabream.

**Keywords**: *Sparus aurata*; vgll3; domestication; SNP variation; gene expressions

# NON-INVASIVE DETECTION OF CIRCULATING MICRORNAS ASSOCIATED WITH COLD STRESS IN TROPICAL AQUACULTURE FISH

Pinhal, D.[1], Barbosa, D.A.[1], Reis Neto, R.V.[2], Hilsdorf, A.W.S.[3], Perazza, C.A.[3] and Campos, V.F.[4]

*[1]Institute of Biosciences, São Paulo State University (UNESP), Botucatu, SP, Brazil; [2]Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Registro, SP, Brazil; [3]Nucleus of Technology and Innovation (NIT), University of Mogi das Cruzes, Mogi das Cruzes, SP, Brazil; [4]Center for Technological Development (CDTec), Federal University of Pelotas, Pelotas, RS, Brazil*

e-mail: danillo.pinhal@unesp.br

Climate change poses increasing challenges for tropical aquaculture species such as tambaqui (*Colossoma macropomum*) and Nile tilapia (*Oreochromis niloticus*), which are adapted to relatively narrow thermal ranges. Identifying molecular biomarkers associated with thermal tolerance is therefore important for improving management strategies and selective breeding programs. In this context, we investigated circulating microRNAs (c-miRNAs) as potential non-invasive biomarkers of cold stress in tropical aquaculture fish. As a preliminary step toward this objective, tambaqui was used as a pilot model to evaluate the feasibility of detecting circulating miRNAs in non-lethal tissues. Blood and mucus samples were collected from three tambaqui subjected to cold stress (21 °C). High-quality RNA obtained from both tissues (RIN > 8; >10 ng/µL) confirmed their suitability for transcriptomic and small RNA analyses. Transcriptomic analysis identified approximately 35,000 transcripts, with more than 20,000 showing consistent expression levels (TPM > 1). Highly expressed genes included ribosomal proteins, metallothioneins, aquaporins, molecular chaperones and components of the ubiquitin–proteasome system, suggesting activation of cellular protection pathways under cold exposure. Small RNA sequencing generated 11.1 million raw reads, yielding 9.7 million high-quality reads after filtering. Most small RNAs ranged between 20–22 nt (>90%), with a dominant peak at 22 nt (~70%), consistent with canonical miRNA processing. A total of 464 precursor miRNAs were identified, including 70 conserved miRNAs from 18 families and several novel predicted candidates. Circulating miRNAs detected in blood and mucus included miR-1, miR-133a-3p and miR-10-5p, which are known to be associated with stress responses and metabolic regulation in teleost fish. Together, these results demonstrate the feasibility of using non-invasive tissues such as blood and mucus for molecular monitoring in aquaculture and highlight circulating microRNAs as promising biomarkers of thermal resilience in tropical aquaculture species.

**Keywords:** non-coding RNAs, aquaculture genomics, biomarkers, transcriptomics

## Posters — Reproduction & improved breeding

# REPRODUCTIVE AXIS DYSREGULATION IN *SOLEA SENEGALENSIS* AQUACULTURE: AN EPIGENOMICS HYPOTHESIS

Carballeda, M.[1]; Torres-Sabino, D.[1]; Aramburu, O.[1]; de la Herrán, R.[2]; Ruiz-Rejón, C.[2]; Robles, F.[2]; Bouza, C.[1] and Martínez, P.[1]

*[1]Departamento de Zoología, Genética y Antropología Física; Facultad de Veterinaria, Universidade de Santiago de Compostela, Lugo, Spain; [2]Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada, Spain*

e-mail: maialen.carballeda.alvarez@usc.es

The Senegalese sole (*Solea senegalensis*) is a high-value flatfish species in European aquaculture, with global farmed production approaching 2,500 tonnes annually and a market value exceeding €19 million per year. As more than 90% of total production relies on aquaculture, the reproductive dysfunction of captive-bred individuals (CB), particularly males, constitutes a major challenge to production efficiency, sustainability and the foreseen expansion (15,000 tons by 2035). The disruption in CB males involves both altered reproductive behaviour, reflected in a deficient courtship, and marked impairment in gametogenesis. Hormonal treatments and environmental enrichment strategies have partially alleviated these failures, although complete recovery of reproductive function has not been achieved. In this work, we integrate transcriptomic and regulatory genomic datasets from the hypothalamus, pituitary and gonad of both male and female individuals from wild and CB origins to investigate molecular signatures associated with the poor reproductive performance under farming conditions. Together, these analyses provide a genomic framework for interpreting reproductive dysfunction in this species by linking alterations in gene expression and regulatory features to reduced reproductive phenotypes. Results will make feasible to develop hatchery protocols or selection (genomic and epigenomic) in Senegalese sole breeding programs. This work advances our understanding of biological regulation under captive conditions and supports future functional studies, and the development of genomics approaches to improve reproductive management in aquaculture.

**Keywords**: Reproductive axis, *Solea senegalensis*, Epigenomics, Aquaculture

# MULTI-OMIC APPROACHES REVEAL SHORT AND LONG-TERM EFFECTS OF HEAWAVE EVENTS IN *ONCORHYNCHUS MYKISS* BREEDERS

Fernández, I. [1], Calvo-Rodríguez, L. [2], de Paz, P. [2] and Riesco, M.F. [2]

[1]*Spanish National Research Council (IGM-CSIC), León, Spain;*
[2]*Universidad de León (ULE), León, Spain.*

e-mail: ignacio.fernandez@ieo.csic.es

Several studies characterized the potential effects of global warming in fish breeders, but little is known about the effects of extreme heatwave (HW) events, the related molecular mechanisms, and how they might be transferred to the resulting offspring. Multi-omic approaches have been conducted to address these questions. Rainbow trout male breeders were exposed or not to a commonly recorded HW in Spain. Hormonal and redox status at blood plasma, sperm quality, and cell proliferation and apoptosis in the testes were assessed in parallel with a mRNA transcriptomic analysis (RNA-Seq). Males exposed to a HW had increased cortisol levels, decreased total antioxidant status and testosterone plasma levels. HW reduced sperm quality, inhibited spermatocyte type I and II differentiation and increased spermatozoa apoptosis. These effects were correlated with the differential expression (DE) of 116 and 1040 (up- and down-regulated, respectively) genes in the testis, reflecting an altered sperm maturation process. We next performed a small non-coding RNA-Seq (sncRNA-Seq) in the same testis samples and identified 8 DE miRs (including miR-221-5p and miR-146-3p). A computational prediction of putative mRNAs targets and its cross-dissection with RNA-Seq results revealed that these DE miRs post-transcriptionally regulate 157 genes. *In vitro* cell transfections using luciferase reporter assays were conducted to validate bioinformatic predictions. Furthermore, although miR-221-5p and miR-146-3p DE was not transferred from the sperm to progenies, miR-221-5p was found DE in the blood plasma of breeders subjected to a HW, being a suitable and less invasive biomarker of reproductive status under HW events. Since miRs does not seem to be the vehicles of transgenerational effects, the DNA methylation profile in progenies was explored. Enzymatic Methyl-sequencing (EM-Seq) analysis from progenies originated from exposed *versus* non-exposed males was performed in the DNA isolated from gills. Differentially methylated regions (DMRs) in different genomic contexts were identified, and potentially downstream regulated genes unveiled, suggesting that DNA methylation is the transgenerational transmission mechanism. Present study not only unveils the potential effects of the extreme events under a climate change scenario, and the underlying mechanisms, but also the potential adaptive mechanisms (DNA methylation) in future farmed fish.

**Keywords**: RNA-Seq, sncRNA-Seq, EM-Seq, Climate change

**Acknowledgements:** REPHEAT (PID2021-127782OA-I00) and MITHEAT (PID2024-160395OB-I00) projects from MICIU/AEI /10.13039/501100011033 and FEDER, UE.

# LEVERAGING A FUNCTIONAL AND EVOLUTIONARY SIGNIFICANCE SCORE FOR VARIANT PRIORITIZATION IN GENOMIC PREDICTIONS OF VNN RESISTANCE IN EUROPEAN SEA BASS

Longo, A. [1], Faggion, S. [1], Babbucci, M. [1], Ferraresso, S. [1], Franch, R. [1] and Bargelloni, L. [1]

[1]*Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (Padova), Italy*

e-mail: alessio.longo.1@phd.unipd.it

Disease outbreaks still represent a serious threat to aquaculture. Conventional selective breeding for complex traits such as disease resistance, relying on estimated breeding values, can be expensive and difficult to implement. In contrast, genomic selection using high-density SNP genotyping provides a more efficient strategy to enhance genetic resistance. Although whole-genome sequencing (WGS) provides opportunities for genomic selection, the inclusion of non-informative variants can introduce noise and increase computational burden. To address this limitation, we used the Functional-And-Evolutionary Trait Heritability (FAETH) score (Xiang et *al*., 2019) to prioritize sequence variants for genomic prediction of viral nervous necrosis resistance (VNN) in European sea bass. A total of 990 juvenile sea bass were phenotyped for body length (mm) and binary VNN mortality after a 29-day VNN experimental challenge test. Parents and 40 experimental fish were whole-genome sequenced (6,072,853 SNPs), enabling WGS imputation for all experimental individuals, genotyped with the Med-Fish SNP array (27,740 SNPs).

The FAETH score was calculated for WGS variants by averaging per-variant heritability estimates for two traits (body length and VNN mortality) across several functional and evolutionary partitions: ATAC-seq/ChIP-seq data, Ensembl annotations, and genomic islands of differentiation between Atlantic and Eastern Mediterranean European sea bass lineages. Variant subsets (1%, 5%, 10%, 25% and 50% SNPs with the highest FAETH scores) were tested in prediction of genomic estimated breeding values for VNN mortality using Bayesian threshold models (GIBBSf90+/BLUPf90+). Eight scenarios, differing in LD-pruning filters (no filtering, 0.99, 0.95 and 0.80 thresholds) and in the inclusion or exclusion of the known major QTL for VNN resistance on chromosome 3 (Mukiibi et *al*., 2025) were considered. Model accuracies were assessed in a 2-fold cross-validation, minimizing genetic relatedness between training and testing sets. In all scenarios, high-ranking variants showed from 2.03% to 8.90% increase in prediction accuracy compared to the bottom 25% low-ranking variants. This study represents the first application of a functional and evolutionary significance score to prioritize SNPs in an aquaculture species, demonstrating its potential to improve genomic prediction accuracy for complex traits.

**Keywords**: *Dicentrarchus labrax*, disease resistance, FAETH-score, WGS data, genomic predictio

**Acknowledgments**: Aqua-faang and EuAqua.org, funded by the European Union, grant n. 817923 and n. 101181589, respectively; PNRR-CN5 Spoke 2 (CUP C93C22002810006).

### *Reference*

Xiang et al. 2019 https://doi.org/10.1073/pnas.1904159116;

Mukiibi et al. 2025 https://doi.org/10.1186/s12915-025-02180-4

# AROMATASE ROLE IN SEX DETERMINATION OF TEMPERATE BASS FISH

Marcos-Hadad, E.[1], Myara, E.[1] and David, L.[1]

[1]*Department of Animal Sciences, RH Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*

e-mail: jennyma@savion.huji.ac.il

Temperate basses (Moronids) are a small group of marine, brackish and freshwater species, from which the European seabass (*Dicentrarchus labrax*) and hybrid striped bass are produced in aquaculture. Hybrid striped bass is produced by crossing males of marine Striped bass (*Morone saxatilis*) with females of freshwater White bass (*Morone chrysops*). We are interested to know what the sex determination mechanisms are in the parental bass species and their hybrid, and their evolutionary conservation, with reference also to European seabass. Unraveling sex determination mechanisms of the parent species, can open doors for developing tools to manipulate sex ratios in the hybrid, which can result in more efficient production of environmentally safer fish. To this day, the sex determination mechanisms of Striped bass and White bass, were unknown. Using genotyping-by-sequencing, we identified a single SEX QTL in Striped bass, and aromatase (*Cyp19a*) as the likely sex determination gene. Males were homozygous to a 19 bp deletion in the second exon, while females were heterozygous, suggesting a ZZ/ZW type mechanism. These genetic findings suggest that this loss of function deletion allows the synthesis of oestradiol from testosterone only in females. We will show further results from a cell culture model on the function of these aromatase alleles. Genotyping-by-sequencing identified three SEX QTLs in White bass suggesting an oligogenic sex determination mechanism, closer to what was previously published for the European seabass. One of these SEX QTLs contains *Cyp19a*, but gene variation was not associated with sex of White bass. Although both females and males were found in hybrid progeny, sex ratios were inconsistent among different progeny groups, suggesting incompatibility in sex determination due to different parental mechanisms. Despite the feasibility of producing hybrid striped bass progeny in captivity, sex determination mechanisms are not well conserved among temperate basses.

**Keywords**: Sex determination gene, ZZ/ZW mechanism*, Morone saxatilis, Morone chrysops*, Hybrid striped bass, Evolutionary conservation

# UNVEILING THE LONG NON-CODING RNA (lncRNA) LANDSCAPE – DIFFERENTIAL EXPRESSION PROFILES IN GILTHEAD SEABREAM GONADS

Papadaki, M. [1], Qi, W. [2], Hotz, A. [2] Mylonas, C.C.M. [1]and Sarropoulou, E. [1]

[1]*Hellenic Center for Marine Research (HCMR), Heraklion, Crete 71003, Greece;*[2]*Functional Genomics Center Zurich, ETH Zurich/University of Zurich, Zurich, Switzerland*

e-mail: mpapadak@hcmr.gr

Long non-coding RNAs (lncRNAs) have been shown to regulate gene expression at the transcriptional and post-transcriptional level through interactions with mRNAs and/or microRNAs (miRNAs). In teleosts, the role of lncRNAs in sex differentiation remains poorly understood. Among aquacultured teleosts, the gilthead seabream *Sparus aurata* is of particular interest as it is a protandrous hermaphrodite that functions as a male for the first two years of life. Afterward, a proportion of fish reverse sex to female. In the reproductive period, fish possess either bisexual gonads (testicular part: mature male, M and ovarian part: immature female, fM) or mature ovaries (F). The present study aims to explore the role of lncRNAs in the M, fM and F gonads of gilthead seabream. Therefore, six fish (three females and three males) aged six years old were sacrificed and the two different parts of the bisexual testis, as well as ovaries of mature females were excised and preserved for RNA extraction. Subsequently, differential expression analysis was carried out. Of the 97,886 assembled transcripts 20,980 were identified as novel lncRNAs. Notably, nearly 40 % (8,157) of the annotated lncRNAs were found to be differentially expressed. Differentially expressed lncRNAs are clustering in two distinct groups corresponding to male (M) and female (fM and F) gonads (Figure 1). Putative lncRNA targets, including the miRNA-mediated interaction between lncRNAs and mRNAs, which reflects the lncRNA/miRNA/mRNA axis, are explored.

**Keywords**: gilthead seabream, lncRNAs, differential expression, gonads, reproduction

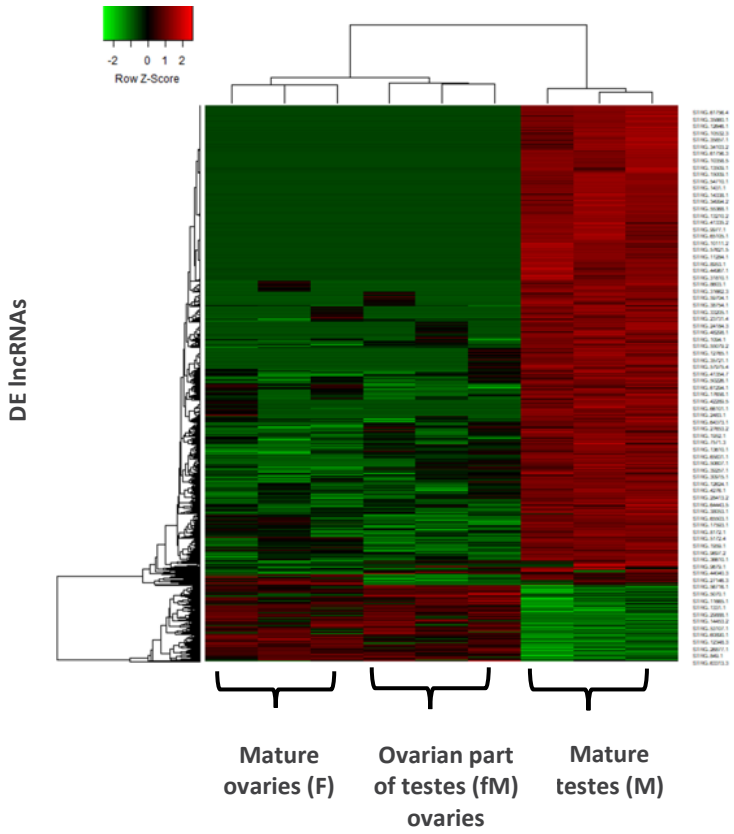



***Figure 1***. *Heatmap of differentially expressed lncRNAs in mature ovaries, ovarian part of testes and the mature testes.*

# TEMPORAL AND MOLECULAR CHARACTERIZATION OF THE MATERNAL-TO-ZYGOTIC TRANSITION (MZT) IN OCTOPUS (*OCTOPUS VULGARIS)*

Guerrero-Peña, L.[1], Pereira, M.[1], González-Pérez, L.[1], Barreiro-Docio, E.[1], García-Fernández, P.[2], Touriñan, P.[2], Chabarrias, D.[2], Saura, M.[1], Rubiolo, J.A.[4] and Rotllant, J.[1*]

*[1]Aquatic Biotechnology Lab. Instituto Investigaciones Marinas-CSIC. Vigo, Spain; [2]Pescanova Biomarine Center, O Grove. Pontevedra. Spain; [3]Universidad de Santiago, Lugo, Spain*

email: rotllant@iim.csic.es

Octopus development is regulated by complex molecular mechanisms that control key developmental transitions, including the maternal-to-zygotic transition (MZT) and the activation of the zygotic genome (ZGA). During this transition, developmental control shifts from maternally deposited RNAs to the transcriptional activity of the embryo's own genome. Despite its importance for early embryogenesis, the molecular mechanisms underlying this process, particularly the interplay between RNA regulation and chromatin accessibility, remain largely unexplored in cephalopods.

In this study, we investigated the molecular and chromatin dynamics associated with the MZT in octopus. Egg–sperm bundles were obtained from cultured octopuses, and embryos were collected at representative developmental stages spanning cleavage, blastulation, gastrulation, and early organogenesis: Stage 1 (fertilized egg), Stage 4 (2 cells, first cleavage), Stage 6 (8 cells, third cleavage), Stage 9 (64–66 cells, sixth cleavage), Stage 10 (blastula), Stage 11 (onset of epiboly), Stage 12 (gastrulation), Stage 18 (early organogenesis with visible mantle, eyes, mouth, and arms), Stage 23 (advanced organogenesis with funnel formation), and Stage 28 (growth stage with developed chromatophores). To characterize transcriptional regulation during these stages, mRNA sequencing and small RNA sequencing were performed to generate transcriptomic profiles across development. These analyses allowed the identification of stage-specific expression patterns of coding RNAs as well as non-coding RNAs, including microRNAs, which are known to contribute to maternal transcript degradation and the activation of the embryonic genome. In parallel, ATAC-seq (Assay for Transposase-Accessible Chromatin sequencing) was used to assess chromatin accessibility and produce a genome-wide map of chromatin compaction and regulatory regions throughout early development. This approach revealed dynamic chromatin remodeling associated with the onset of zygotic transcription. Together, these results provide the first integrated characterization of RNA expression dynamics and chromatin accessibility during the MZT in octopus, offering new insights into the molecular regulation of early development in cephalopods and establishing a foundation for future studies in developmental biology and octopus aquaculture.

**Keywords**: Octopus, MZT, ZGA, RNA-seq, ATAC-seq

**Acknowledgements:** This research was funded by the MCIN/AEI/10.13039/501100011033 grant number PID2024-161686OB-100 and CSIC intramural project PIE-202340E076 to JR and by "ERDF A way of making Europe"

# GENOME-WIDE EPIGENETIC ALTERATIONS INDUCED BY SPERM CRYOPRESERVATION IN NILE TILAPIA

Rudnik, L. M.[1], Hidalgo, M. M. T.[2], Cecconello, D. M.[3], Appel, R. C.[4], Silva, V. W.[3], Tripathy, P. S.[5], Vilas-Boas, L. A.[3], Giordano, L. G. P.[3], Martins, M. I. M.[3], Paschoal, A. R.[1,6] and Fernandes, J. M. O.[4]

*[1] Federal Technological University of Paraná, Brazil, [2]State University of Northern Paraná, Brazil, [3] State University of Londrina, Brazil, [4]Institute of Marine Sciences (ICM-CSIC), Spain, [5]Nord University, Norway, [6]Rosalind Franklin Institute, United Kingdom*

e-mail: lorenarudnik43@gmail.com

Cryopreservation is a fundamental biotechnological tool in aquaculture, as it enables the establishment of cryobanks for long-term storage of genetic material from superior strains. However, the safe application of this biotechnology requires an understanding that germline cells transmit not only genetic information to the embryo but also epigenetic marks, such as DNA methylation, which may be susceptible to alterations during the freezing and thawing processes. This is currently a limitation for genetic improvement programs in Nile tilapia (*Oreochromis niloticus*). The aim of this study was to evaluate the impact of sperm cryopreservation on the DNA methylome in Nile tilapia. Semen samples were obtained from six Nile tilapia males maintained in a recirculating aquaculture system and analyzed i) immediately after collection or ii) following cryopreservation and thawing (Silva et al., 2025). Analysis of sperm quality parameters revealed a significant reduction in total motility and an increase in morphological abnormalities following cryopreservation ($P < 0.001$). Genomic DNA was extracted from both groups for preparation and sequencing of reduced representation bisulfite sequencing (RRBS) libraries. Bioinformatic analyses were performed in R using the methylKit package to compare DNA methylation profiles between fresh and cryopreserved sperm. Numerous differentially methylated cytosines were identified with cryopreservation across the genome. Understanding these cryopreservation-induced epigenetic changes is essential for the safe application of reproductive biotechnologies in aquaculture.

**Keywords:** DNA methylation, bioinformatics, epigenetics, sperm cryopreservation

**Acknowledgements:** This study received financial support from the European Research Council (project nos. 683210 and 101081935), the Research Council of Norway (project no. 310103), the Spanish Ministry of Science and Innovation (grant PID2023-146286OB-I00 “Holosex”; grant CEX2024-001494-S funded by AEI 10.13039/501100011033), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, PDSE Program (nº 26/2024).

**Reference:** Silva, V. W., Hidalgo, M. M. T., Appel, R. J. C., Siqueira, K. N., Beirão, J., Vilas-Boas, L. A., Pretto-Giordano, L. G., Joshi, R., Fernandes, J. M. O., & Martins, M. I. M. (2025). Influence of extenders and antioxidants on Nile tilapia (*Oreochromis niloticus*) sperm parameters. Cryobiology, 119, 105253.

# UNCOVERING GENETIC VARIATION IN PATHOGEN SUSCEPTIBILITY: A MULTI-OMICS APPROACH IN SENEGALESE SOLE AQUACULTURE

Silva, I.A.L.[1], Freitas, I.[1], Angelo, M.[1], Gregório, S.[1], Sousa, R.[1], Pousão-Ferreira, P. [1,2], Medina, D.[3], Castro, C.[3], Soares, F.[1,2] and Lourenço-Marques, C. [1,2]

[1] S2AQUA, Olhão, Portugal, [2] IPMA/EPPO, Olhão, Portugal, [3] FLATLANTIC Lta., Mira, Portugal.

e-mail: iris.silva@s2aquacolab.pt

Meeting the rising need for sustainable seafood production requires innovative strategies to strengthen the resilience of key aquaculture species. Senegalese sole (*Solea senegalensis*) remains highly valued in European markets but vulnerable to disease due to its close interaction with sediment environments. Within this context, the BETTERFLAT project explores how integrated genomic tools can support the development of disease-resistant stocks by combining high-throughput molecular approaches, including transcriptomics, functional genomics, and marker discovery, to uncover the biological basis of host–pathogen interactions. The first steps of this work involved refining infection protocols through preliminary challenge trials, where lethal dose thresholds were established and used to standardize experimental conditions. Particular attention was given to infections caused by *Photobacterium damselae piscicida*, a major bacterial threat in marine aquaculture, revealing significant challenges linked to host variability. The study further incorporated pathogen molecular characterization as well as molecular assessment of virulence genes in the strain used in the challenge trials. When individuals from four distinct families, each representing a different genetic background, were exposed to the same bacterial concentration corresponding to the LD75, markedly different mortality patterns were observed, highlighting substantial genetic influence on disease susceptibility. These findings emphasize the complexity of selecting for resistance, as uniform pathogen exposure does not yield uniform outcomes across genetically diverse populations. By integrating phenotypic responses from challenge tests with multi-omics data, this work advances the identification of candidate markers associated with resistance traits. Ultimately, these insights contribute to the establishment of selective breeding programs aimed at improving robustness and survival in Senegalese sole, while also addressing broader challenges in aquaculture related to disease control, production efficiency, and environmental sustainability.

**Keywords**: Sole, RNA-Seq, disease resistance, transcriptomics, genomics, bacterial challenge

**Acknowledgments:** The operation BetterFLAT was developed with funding from Programmes COMPETE2030, Algarve2030, by Portugal2030 and the European Union, Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022. P148) and SAUDE&AQUA II (MAR-021.1.3-FEAMPA-0001.

## Posters — Low trophic aquaculture

# GENOMICS-BASED IDENTIFICATION OF MICRO- AND MACROALGAE: ADVANCING BIOBANKING AND AQUACULTURE APPLICATIONS

Silva, I.A.L. [1*], Angelo, M.[1*], Freitas, I.[1], Quintã, R. [1], Gonçalves, C.[1], Pousão-Ferreira, P.[1,2] and Lourenço-Marques, C.[1,2]

[1] S2AQUA, Olhão, Portugal, [2] IPMA/EPPO, Olhão, Portugal; *Contributed equally.

e-mail: iris.silva@s2aquacolab.pt

Accurate species identification is essential for biodiversity research, aquaculture, and biobank management. S2AQUA offers a molecular identification service specializing in macro- and microalgae, using a workflow based on automated DNA extraction, polymerase chain reaction (PCR), sequencing, and phylogenetic analysis. This approach enables precise species determination, addressing the lack of traditional morphological expertise, which can be time-consuming to gain and apply. This efficient method is particularly beneficial for biobanks, research institutions, aquaculture facilities, and algae related industry, where accurate species identification is crucial for numerous applications, including use for food, feed, and pharmaceuticals. Once the sample is received in S2AQUA's labs, the genomic DNA is extracted by an automated system, quantified and using species-specific primers, PCR is performed by amplifying targets key genetic markers, for instance the *ITS1* for *Ulva sp.* and *Laminaria ochroleuca* or *cox1* for *Gracilaria gracilis*. These genes are highly conserved between species yet variable enough for precise specie identification. The amplified DNA is then sent for sequencing, and the sequence is compared against reference databases for phylogenetic analysis and accurate taxonomic classification. So far, species from Chlorophyta, Rhodophyta, and Heterokontophyta phyla, including *Ulva ohnoi*, *Ulva mutabilis*, *Codium fragile*, *Codium tomentosum*, *Codium decorticatum*, *Chondrus crispus*, *Gracilaria gracilis*, *Gracilariopsis longissima*, *Laminaria ochroleuca*, *Saccharina latissima*, *Saccorhiza polyschides*, *Dunaliella salina*, and *Nannochloropsis oceanica*, have been successfully identified. These results demonstrate the effectiveness and reliability of the applied molecular identification methodology. All these analyses can be tailor-made upon request. The molecular identification workflow used by S2AQUA is a reliable, rapid and efficient method for classifying macro- and microalgae. The successful identification of multiple species demonstrates its effectiveness for use in different sectors and enhances species traceability. Ultimately, it supports resource optimization and strengthens scientific and industrial applications requiring precise species classification.

**Keywords**: Molecular identification, macroalgae, microalgae, biobanking

**Acknowledgments:** This study had the support of the projects WP9- Portuguese Blue Biobank under the Blue Economy Pact - Project Nº. C644915664-00000026, and Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148).

# PRESENTING AUTHOR INDEX

| AUTHOR (PRESENTING) | TITLE | INSTITUTION | PRESENTATION TYPE | PAGE |
|---|---|---|---|---|
| Silva, I.A.L. | IDENTIFICATION OF NEW KEY STRESS RELATED GENES BY COMPARATIVE ANALYSIS OF THE GENE EXPRESSION PROFILE UNDER MULTIPLE ENVIRONMENTAL STRESSORS IN GILTHEAD SEABREAM (*SPARUS AURATA*) | S2AQUA | Oral | 21 |
| Lazado, C.C. | CIRCADIAN REGULATION OF IMMUNE FUNCTIONS IN ATLANTIC SALMON LEUKOCYTES | Nofima | Oral | 22 |
| López-Chillarón, S. | SEX-DEPENDENT REGULATION OF IMMUNE RESPONSES IN EUROPEAN SEA BASS GONADS | ICM-CSIC | Oral | 23 |
| **Session 3 — Immunity, stress & welfare III** | | | | |
| Veiga-Rúa, S. | DECODING THE GENETIC BASIS OF FISH IMMUNE RESPONSES USING KNOCKOUT CELL LINES | University of Santiago de Compostela | Oral | 24 |
| Robinson, N. | HOW COULD GENOMIC KNOWLEDGE HELP MAKE ATLANTIC SALMON MORE “COHO-LIKE” IN THEIR ABILITY TO RESIST SEA LICE? | Nofima / Deakin University | Oral | 25 |
| Valenzuela-Muñoz, V. | EPITRANSCRIPTOMIC REGULATION OF ATLANTIC SALMON DURING *CALIGUS ROGERCRESSEYI* INFESTATION | University of Concepción | Oral | 26 |
| Iversen, M. | DIFFERENTIAL GILL TRANSCRIPTOME RESPONSES IN ATLANTIC SALMON TO CLONAL AND REISOLATED CULTURES OF *NEOPARAMOEBA PERURANS*, AGENT OF AMOEBIC GILL DISEASE | Nofima | Oral | 27 |
| Pereira, M. | CIRCULATING MICRORNAS AS EMERGING BIOMARKERS FOR STRESS MONITORING IN *OCTOPUS VULGARIS* AQUACULTURE | IIM-CSIC | Oral | 28 |
| Aramburu, O. | UNCOVERING THE MICRORNA REGULATORY LANDSCAPE OF TURBOT | University of Santiago de Compostela / GENEAQUA S.L. | Oral | 29 |
| **Session 4 — Nutrition & growth** | | | | |
| Navarro-Guillén, C. | DIET SHAPES FUNCTIONAL HOST–MICROBIOTA ASSOCIATIONS IN GILTHEAD SEABREAM REVEALED BY INTEGRATED TRANSCRIPTOME AND MICROBIOTA PROFILES | ICMAN-CSIC | Oral | 31 |
| Lourenço-Marques, C. | TRANSCRIPTOMIC PROFILING OF FAST- AND SLOW-GROWING *SPARUS AURATA* REVEALS POTENTIAL GROWTH BIOMARKERS | S2AQUA | Oral | 32 |
| Sarropoulou, E. | PHYTOGENIC STRATEGIES TO ENHANCE GUT RESILIENCE IN SEABREAM | HCMR | Oral | 33 |
| Perera, E. | MILD OXIDATIVE STRESS GUIDES DIETARY EPIGENETIC MODULATORS TO DIRECT DNA METHYLATION TOWARD STRESS-RESILIENCE PATHWAYS | ICMAN-CSIC | Oral | 34 |
| Ecker-Eckhofen, E. | EPIGENETIC BASIS OF THE ONSET OF SEXUAL SIZE DIMORPHISM IN TELEOSTS | ICM-CSIC | Oral | 35 |
| Sobhiafshar, U. | SINGLE-NUCLEUS TRANSCRIPTOMICS OF ATLANTIC SALMON MUSCLE CAPTURES MYOGENIC CELL STATES AND POST-DUPLICATION DIVERGENCE | The Roslin Institute | Oral | 36 |
| **Session 5 — Microbial genomics** | | | | |
| Duchaud, E. | *FLAVOBACTERIUM PSYCHROPHILUM* GENOMICS TO UNRAVEL EVOLUTION, VIRULENCE AND HOST ADAPTATION OF A MAJOR FISH PATHOGEN | INRAE | Oral | 37 |

| AUTHOR (PRESENTING) | TITLE | INSTITUTION | PRESENTATION TYPE | PAGE |
|---|---|---|---|---|
| Saidan, L. | IDENTIFICATION OF AN ANTIBIOTIC RESISTANCE CLUSTER IN *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA* ASSOCIATED WITH NEW PLASMIDS AND A-LAYER EXPRESSION | INRAE | Oral | 38 |
| Silva, D. | PHOTOPERIOD-DRIVEN SEX-SPECIFIC SHIFTS IN BACTERIAL AND FUNGAL GUT COMMUNITIES IN ATLANTIC COD (*GADUS MORHUA*) | ICM-CSIC | Oral | 39 |
| Abdollahpour, H. | THERMAL AND DEVELOPMENTAL DRIVERS OF MICROBIAL COMMUNITY DYNAMICS IN GILL, INTESTINE AND SKIN OF GILTHEAD SEA BREAM AND EUROPEAN SEABASS DURING EARLY ONTOGENY | CCMAR | Oral | 40 |
| Overland, B. | EFFECTS OF A DIETARY BACTERIOPHAGE TARGETING *FLAVOBACTERIUM PSYCHROPHILUM* ON THE GUT MICROBIOME OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) | Swansea University | Oral | 41 |
| Uren Webster, T. | PRIMING THE FISH MICROBIOME IN EARLY LIFE TO IMPROVE STRESSOR RESILIENCE. | Swansea University | Oral | 42 |
| **Session 6 — Integrative omics** | | | | |
| Allal, F. | GENOME ARCHITECTURE SHAPES EPIGENETIC AND TRANSCRIPTIONAL DIVERGENCE IN EUROPEAN SEABASS POPULATIONS | MARBEC, Ifremer | Oral | 44 |
| Konstantinidis, I. | CELL-TYPE-SPECIFIC ENDOCRINE SIGNALS OF THE PITUITARY GLAND IN RESPONSE TO PHOTOPERIOD MANIPULATION | Nord University | Oral | 45 |
| Colonna, L. | PHOTOPERIOD EFFECT ON ATLANTIC COD GONADS AND PITUITARY REVEALS MODULATION OF GENE EXPRESSION AND METHYLATION | Nord University | Oral | 46 |
| Vera, M. | INTEGRATIVE OMICS ANALYSES REVEAL GENOMIC DIFFERENTIATION AND DIVERGENT GENE EXPRESSION ACROSS BROWN TROUT LINEAGES | University of Santiago de Compostela | Oral | 47 |
| Rodriguez-Barreto, D. | METHODOLOGICAL INSIGHTS FOR STUDYING LNCRNAS IN FISH: BENCHMARKING TRANSCRIPTOME DEPTH AND EXTRACELLULAR VESICLE RNA PROFILING | University of La Laguna | Oral | 48-49 |
| Clark, T.C. | SHELL-ULAR BIOLOGY: SINGLE NUCLEI-RESOLVED GENE EXPRESSION CHANGES IN THE EPITHELIAL TISSUE OF MOULTING *LITOPENAEUS VANNAMEI* | The Roslin Institute, | Oral | 50 |
| Buha, T. | SKIN MUCUS AND TISSUE MULTIOMICS COMPARISON OF TRANSPORT-INDUCED ACUTE STRESS RESPONSES IN ATLANTIC SALMON, EUROPEAN SEABASS AND RAINBOW TROUT | CIIMAR, ICBAS and SPAROS | Oral | 51 |
| **Session 7 — Reproduction & improved breeding I** | | | | |
| Palaiokostas, C. | PRECISION GENOMICS IN ARCTIC CHARR: CUSTOM SNP-CHIPS FOR BACTERIAL KIDNEY DISEASE RESISTANCE AND DIVERSITY MANAGEMENT | Swedish University of Agricultural Sciences | Oral | 52 |
| Jones, D.B. | COMPLEXITIES OF SELECTIVE BREEDING WITHIN PEARL OYSTERS — A LONGITUDINAL STUDY REVEALS MOLECULAR DRIVERS OF SHELL GROWTH AND COLOUR | James Cook University | Oral | 53 |
| Allal, F. | THE SPERM RACE MATTERS: PHENOTYPIC AND GENOMIC EVIDENCE FOR WITHIN-EJACULATE HAPLOID SELECTION IN *DICENTRARCHUS LABRAX* | MARBEC, Ifremer | Oral | 54 |

| AUTHOR (PRESENTING) | TITLE | INSTITUTION | PRESENTATION TYPE | PAGE |
|---|---|---|---|---|
| Torres-Sabino, D. | TRANSCRIPTOMIC AND EPIGENETIC CHANGES IN THE OLFACTORY ORGANS OF SENEGALESE SOLE MAY UNDERLIE REPRODUCTIVE FAILURE IN CAPTIVE-BRED MALES | University of Santiago de Compostela | Oral | 55 |
| Żarski, D. | OVULATION DEFINES FINAL MATERNAL RNA LANDSCAPE THROUGH EXTENSIVE TRANSCRIPTOMIC REMODELING IN PIKEPERCH EGGS | InLife Institute | Oral | 56 |
| Ocalewicz, K. | TRANSCRIPTOMICS OF RAINBOW TROUT EGGS USED FOR GYNOGENESIS: PROTECTION FROM PHYSICAL STRESS VS. SENSITIVITY OF MICROTUBULES FOR HYDROSTATIC PRESSURE SHOCK | University of Gdansk | Oral | 57 |
| **Session 8 — Reproduction & improved breeding II** | | | | |
| Ribas, L. | ROOTING THE FUTURE OF SPACE AQUACULTURE | ICM-CSIC | Oral | 58 |
| Aslam, M. L. | SHARED GENETIC ARCHITECTURE REVEALS PLEIOTROPIC QTL(S) REGULATING GONADOSOMATIC INDEX AND SEXUAL MATURITY IN ATLANTIC COD | Nofima | Oral | 59 |
| Mengesha, T. | REPRODUCTIVE PERFORMANCE OF ZEBRAFISH (*DANIO RERIO*) CRISPR/CAS9 MUTANT LINES | University of Gondar | Oral | 60 |
| **Session 8 — Low trophic aquaculture** | | | | |
| Chapuis, A. | EXPLORING GENETIC DIVERSITY AND HYBRIDISATION PATTERNS OF MUSSELS IN NORTHERN SCOTLAND AND IRELAND | The Roslin Institute | Oral | 61-62 |
| Regan, T. | LONG-READ SEQUENCING REVEALS EXTENSIVE STRUCTURAL VARIATION IN FARMED SCOTTISH *MYTILUS* MUSSELS | The Roslin Institute | Oral | 63 |
| Gallo, M. | FROM CHALLENGE TEST TO GWAS: UNCOVERING GENOMIC SIGNALS OF SALINITY TOLERANCE IN THE COMMON COCKLE | University of Santiago de Compostela | Oral | 64 |
| **POSTER PRESENTATIONS** | | | | |
| **Posters — Immunity, stress & welfare** | | | | |
| Arana, A.J. | FUNCTIONAL VALIDATION OF IFI27L2A AS A RESTRICTION FACTOR FOR NNV REPLICATION | University of Santiago de Compostela | Poster | 65 |
| Bastos, B. | VITTELOGENINS EXCLUSION DURING MASS SPECTROMETRY DATA ACQUISITION ENHANCES PROTEIN CO-EXPRESSION ANALYSIS IN EGGS FROM PATHOGEN CHALLENGED ZEBRAFISH | CIIMAR | Poster | 66 |
| Budik, S. | NUCLEOTIDE SEQUENCE BASED EPIDEMIOLOGICAL SURVEILLANCE IS ESSENTIAL FOR TRACKING VHS OUTBREAKS IN AUSTRIAN TROUT AQUACULTURE | Veterinärdirektion Land Steiermark | Poster | 67 |
| Casuso, A. | VACCINATION AT DIFFERENT LIFE STAGES DETERMINES THE IMMUNE RESPONSE TO *PISCIRICKETTSIA SALMONIS* IN ATLANTIC SALMON: A TRANSCRIPTOME ANALYSIS | University of Concepción | Poster | 68-69 |
| Chaiyasut, K. | DIVERGENT HEALING TRAJECTORIES AMONG MECHANICAL SKIN WOUND TYPES IN POST-SMOLT ATLANTIC SALMON (*SALMO SALAR*) | The Institute of Aquaculture, University of Stirling | Poster | 70 |

| AUTHOR (PRESENTING) | TITLE | INSTITUTION | PRESENTATION TYPE | PAGE |
|---|---|---|---|---|
| Martínez, P. | POPULATION / INDIVIDUAL GENOMIC ASSOCIATION AND TRANSCRIPTOMIC DATA SUPPORTS A LONG INVERSION IN CHROMOSOME 8 UNDERLYING RESISTANCE OF THE EUROPEAN FLAT OYSTER (*OSTREA EDULIS*) TO *BONAMIA OSTREAE* | University of Santiago de Compostela | Poster | 71 |
| Pérez-Pereira, N. | EVOLUTION OF THE TRIM FAMILY OF E3 UBIQUITIN LIGASES IN TELEOST FISH | University of Santiago de Compostela | Poster | 72 |
| Rudnik, L.M. | PROLONGED LIGHT STRESS INFLUENCES EXPRESSION OF TRANSPOSABLE ELEMENTS IN *GADUS MORHUA* | Federal Technological University of Paraná | Poster | 73 |
| Tengs, T. | THE EFFECTS OF HEATWAVES ON ATLANTIC SALMON INFECTED WITH *YERSINIA RUCKERI* | Nofima | Poster | 74 |
| **Posters — Nutrition & growth** | | | | |
| Moulistanos, A. | FUNCTIONAL MODULATION OF GUT BACTERIAL COMMUNITIES IN GILTHEAD SEABREAM BY DIETARY ASTAXANTHIN | Aristotle University of Thessaloniki | Poster | 75 |
| Oikonomou, S. | ENDOCRINE GENE EXPRESSION ASSOCIATED WITH GROWTH AND LIPID DEPOSITION UNDER ALTERNATIVE DIETS IN GILTHEAD SEABREAM | International Hellenic University | Poster | 76 |
| Viswanath, K. | HALOPHYTE EXTRACTS TEMPER THE FUNCTIONS OF ATLANTIC SALMON HEAD KIDNEY ADHERENT LEUKOCYTES AND GILL EPITHELIAL CELLS | Nord University | Poster | 77 |
| Viswanath, K. | *LACTOCOCCUS* LACTIS AND (1,3)-β-GLUCAN DRIVE REMODELING OF IMMUNE RESPONSES AND MICROBIAL METABOLIC PROFILE IN ZEBRAFISH | Nord University | Poster | 78 |
| **Posters — Microbial genomics** | | | | |
| James, L. | NOVEL QPCR ASSAY OPTIMISED FOR ANALYSIS OF PHAGE THERAPY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND RAMIFICATIONS FOR GUT MICROBIOME | Swansea University | Poster | 79 |
| Sandoval, A. | IN SILICO CHARACTERIZATION OF TYPE VI SECRETION SYSTEMS IN EM-90 AND LF-89 GENOGROUPS OF *PISCIRICKETTSIA SALMONIS* | University of Concepción | Poster | 80 |
| Silva, I.A.L. | ADVANCED PCR-BASED TOOLS FOR EARLY PATHOGEN IDENTIFICATION AND DISEASE MANAGEMENT IN AQUACULTURE | S2AQUA | Poster | 81 |
| Trumbić, Ž. | EXPLORING MUSSEL MICROBIOMES IN THE KRKA RIVER ESTUARY | University of Split | Poster | 82 |
| **Posters — Integrative omics** | | | | |
| Appel, R.J.C. | EXPLORING INTERGENERATIONAL EPIGENETIC EFFECTS IN ATLANTIC SALMON AQUACULTURE: THE EPIBROOD PROJECT | ICM-CSIC | Poster | 83 |
| Chapman, A.L. | CRUDE OIL-INDUCED EPIGENETIC ALTERATIONS IN THE POLAR COD (*BOREOGADUS SAIDA*) REPRODUCTIVE AXIS: INSIGHTS FROM EM-SEQ | The Arctic University of Norway | Poster | 84 |
| Chapman, A.L. | OYSTER: EMPOWERING EARLY CAREER RESEARCHERS IN MARINE SCIENCE ACROSS EUROPE | OYSTER (Orienting Young ScienTists of EuromaRine) | Poster | 85 |
| Gonzalez-Prendes, R. | GENOME-WIDE IDENTIFICATION OF LOCI AND IMMUNE-RELATED CANDIDATE GENES FOR RESISTANCE TO SALMON RICKETTSIAL SYNDROME IN ATLANTIC SALMON | Hendrix Genetics B.V. | Poster | 86 |

| AUTHOR (PRESENTING) | TITLE | INSTITUTION | PRESENTATION TYPE | PAGE |
|---|---|---|---|---|
| Leal, Y. | TRANSCRIPTOMIC INSIGHTS INTO ATLANTIC SALMON IMMUNE RESPONSES TO *PISCIRICKETTSIA SALMONIS* GENOGROUPS | University of Concepción | Poster | 87 |
| Manousi, D. | A MAJOR LOCUS ON CHROMOSOME 14 IMPACTS DEVELOPMENTAL VARIATION OF ATLANTIC SALMON SMOLTIFICATION | Norwegian University of Life Sciences / Nofima | Poster | 88 |
| Mehta, T. | EPIGENETIC AND TRANSCRIPTOMIC RESILIENCE OF FISH EMBRYOS FOR FUTURE SPACE AQUACULTURE | University of Liverpool | Poster | 89 |
| Moulistanos, A. | INTEGRATING GENOMIC AND TRANSCRIPTOMIC ANALYSES REVEALS A KEY REGULATORY ROLE OF VGLL3 IN GILTHEAD SEABREAM DOMESTICATION | Aristotle University of Thessaloniki | Poster | 90 |
| Pinhal, D. | NON-INVASIVE DETECTION OF CIRCULATING MicroRNAs ASSOCIATED WITH COLD STRESS IN TROPICAL AQUACULTURE FISH | São Paulo State University | Poster | 91 |
| **Posters — Reproduction & improved breeding** | | | | |
| Carballeda, M. | REPRODUCTIVE AXIS DYSREGULATION IN *SOLEA SENEGALENSIS* AQUACULTURE: AN EPIGENOMICS HYPOTHESIS | University of Santiago de Compostela | Poster | 92 |
| Fernández, I. | MULTI-OMIC APPROACHES REVEAL SHORT AND LONG-TERM EFFECTS OF HEATWAVE EVENTS IN *ONCORHYNCHUS MYKISS* BREEDERS | IGM-CSIC | Poster | 93 |
| Longo, A. | LEVERAGING A FUNCTIONAL AND EVOLUTIONARY SIGNIFICANCE SCORE FOR VARIANT PRIORITIZATION IN GENOMIC PREDICTIONS OF VNN RESISTANCE IN EUROPEAN SEA BASS | University of Padova | Poster | 94 |
| Marcos-Hadad, E. | AROMATASE ROLE IN SEX DETERMINATION OF TEMPERATE BASS FISH | The Hebrew University of Jerusalem | Poster | 95 |
| Papadaki, M. | UNVEILING THE LONG NON-CODING RNA (LNCRNA) LANDSCAPE — DIFFERENTIAL EXPRESSION PROFILES IN GILTHEAD SEABREAM GONADS | HCMR | Poster | 96 |
| Rotllant, J. | TEMPORAL AND MOLECULAR CHARACTERIZATION OF THE MATERNAL-TO-ZYGOTIC TRANSITION (MZT) IN OCTOPUS (*OCTOPUS VULGARIS*) | IIM-CSIC | Poster | 97 |
| Rudnik, L.M. | GENOME-WIDE EPIGENETIC ALTERATIONS INDUCED BY SPERM CRYOPRESERVATION IN NILE TILAPIA | Federal Technological University of Paraná | Poster | 98 |
| Silva, I.A.L. | UNCOVERING GENETIC VARIATION IN PATHOGEN SUSCEPTIBILITY: A MULTI-OMICS APPROACH IN SENEGALESE SOLE AQUACULTURE | S2AQUA | Poster | 99 |
| **Posters — Low trophic aquaculture** | | | | |
| Silva, I.A.L. | GENOMICS-BASED IDENTIFICATION OF MICRO- AND MACROALGAE: ADVANCING BIOBANKING AND AQUACULTURE APPLICATIONS | S2AQUA | Poster | 100 |

# GIA 2026 | SPLIT, 4–6 MAY

# PROGRAMME

| DAY 1 — MONDAY, 4 MAY 2026 | | | |
|---|---|---|---|
| **Time** | **Title** | **Presenting author** | **Institution** |
| **08:30** | ***REGISTRATION*** | | |
| **09:15** | **GIA WELCOME — Opening speeches** | | |
| **09:45** | **PLENARY LECTURE — Gen Hua Yue (Temasek Life Sciences Laboratory, Singapore)** | | |
| **~10:30** | ***Coffee break*** | | |
| **Session 1: Immunity, stress & welfare I** | | **Chairs: Lior David, Carlo Lazado** | |
| 11:00 | LONG-READ GRAPH GENOME OF SEA BREAM HIGHLIGHTS STRUCTURAL VARIANT ENRICHMENT IN GENES DRIVING ADAPTIVE PLASTICITY | Aldo Hernández-Juarez | IATS-CSIC |
| 11:15 | RNA SEQUENCING REVEALS MARKERS OF IMMUNITY IN ATLANTIC SALMON (SALMO SALAR) VACCINATED AGAINST TENACIBACULUM FINNMARKENSE | Alexandra Florea | The Roslin Institute |
| 11:30 | DISEASE RESISTANCE MECHANISMS AND INFECTIVITY ARE DIFFERENT BETWEEN COMMON CARP AND RELATED CYPRINIDS | Lior David | Hebrew Univ. Jerusalem |
| 11:45 | INSIGHTS INTO THE GENETIC ARCHITECTURE OF RESISTANCE TO VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN RAINBOW TROUT FROM GWAS AND FUNCTIONAL VALIDATION | Valentin Thomas | INRAE |
| 12:00 | MICRORNA-MEDIATED REGULATION OF HOST RESPONSE TO VIRAL NERVOUS NECROSIS VIRUS IN EUROPEAN SEABASS | Raquel Rodríguez-Vázquez | Univ. Santiago de Compostela |
| 12:15 | CRISPR-CAS9 EDITING AS A TOOL TO INTERROGATE GENE FUNCTION: ATLANTIC SALMON TRIM25 PARALOGS ANTI-VIRAL FUNCTION IN INFECTIOUS SALMON ANAEMIA VIRUS | Robert Stewart | The Roslin Institute |
| 12:30 | Lunch break | | |
| **Session 2: Immunity, stress & welfare II** | | **Chairs: Robert Stewart, Jerko Hrabar** | |
| 14:30 | EARLY-LIFE CONDITIONING INFLUENCES LATER-LIFE STRESS RESPONSES IN RAINBOW TROUT: INSIGHTS FROM MULTI-OMICS ANALYSES | Arun Shankregowda | Swansea University |
| 14:45 | THE EMBRYONIC ENVIRONMENT: KEY TO ROBUSTNESS IN ATLANTIC SALMON | Erik Burgerhout | Nofima |
| 15:00 | IDENTIFICATION OF FISH EPIGENETIC BIOMARKERS OF DISEASE RESISTANCE DRIVEN BY EARLY REARING CONDITIONS | Sofia Consuegra | Swansea University / IIM-CSIC |
| 15:15 | CHALLENGES AND CONSIDERATIONS FOR NANOPORE DNA METHYLATION SEQUENCING IN ATLANTIC SALMON | Ethan Friis | Swansea University |
| 15:30 | GENOMIC EVIDENCE FOR CONSERVED ENVIRONMENTAL SENSING REGULATORS UNDERLYING DOMESTICATION AND BREEDING-RELATED DIVERGENCE IN GILTHEAD SEABREAM (SPARUS AURATA) | Aristotelis Moulistanos | Aristotle Univ. Thessaloniki |
| 15:45 | IDENTIFICATION OF NEW KEY STRESS RELATED GENES BY COMPARATIVE ANALYSIS OF THE GENE EXPRESSION PROFILE UNDER MULTIPLE ENVIRONMENTAL STRESSORS IN GILTHEAD SEABREAM (SPARUS AURATA) | Iris Silva | S2AQUA |
| 16:00 | CIRCADIAN REGULATION OF IMMUNE FUNCTIONS IN ATLANTIC SALMON LEUKOCYTES | Carlo Lazado | Nofima |
| 16:15 | SEX-DEPENDENT REGULATION OF IMMUNE RESPONSES IN EUROPEAN SEA BASS GONADS | Sandra López-Chillarón | ICM-CSIC |
| **16:30** | **ICE BREAKER & POSTER SESSION** | | |
| **18:30** | **End of Day 1** | | |

| DAY 2 — TUESDAY, 5 MAY 2026 | | | |
|---|---|---|---|
| **Time** | **Title** | **Presenting author** | **Institution** |
| **09:45** | **PLENARY LECTURE — Kaja Helvik Skjærven (Institute of Marine Research, Norway)** | | |
| **~10:30** | ***Coffee break*** | | |
| **Session 3: Immunity, stress & welfare III** | | **Chairs: Sofia Consuegra, Tim Regan** | |
| 11:00 | DECODING THE GENETIC BASIS OF FISH IMMUNE RESPONSES USING KNOCKOUT CELL LINES | Sara Veiga-Rúa | Univ. Santiago de Compostela |
| 11:15 | HOW COULD GENOMIC KNOWLEDGE HELP MAKE ATLANTIC SALMON MORE “COHO-LIKE” IN THEIR ABILITY TO RESIST SEA LICE? | Nick Robinson | Nofima / Deakin University |
| 11:30 | EPITRANSCRIPTOMIC REGULATION OF ATLANTIC SALMON DURING CALIGUS ROGERCRESSEYI INFESTATION | Valentina Valenzuela-Muñoz | Univ. de Concepción |
| 11:45 | DIFFERENTIAL GILL TRANSCRIPTOME RESPONSES IN ATLANTIC SALMON TO CLONAL AND REISOLATED CULTURES OF NEOPARAMOEBA PERURANS, AGENT OF AMOEBIC GILL DISEASE | Marianne Iversen | Nofima |
| 12:00 | CIRCULATING MICRORNAS AS EMERGING BIOMARKERS FOR STRESS MONITORING IN OCTOPUS VULGARIS AQUACULTURE | Mariana Pereira | IIM-CSIC |
| 12:15 | UNCOVERING THE MICRORNA REGULATORY LANDSCAPE OF TURBOT | Oscar Aramburu | Univ. Santiago de Compostela / GENEAQUA |
| ***12:30*** | ***Lunch break*** | | |
| **Session 4: Nutrition & growth** | | **Chairs: Jorge Fernandes, Ivana Lepen Pleić** | |
| 14:30 | DIET SHAPES FUNCTIONAL HOST–MICROBIOTA ASSOCIATIONS IN GILTHEAD SEABREAM REVEALED BY INTEGRATED TRANSCRIPTOME AND MICROBIOTA PROFILES | Carmen Navarro-Guillén | ICMAN-CSIC |
| 14:45 | TRANSCRIPTOMIC PROFILING OF FAST- AND SLOW-GROWING SPARUS AURATA REVEALS POTENTIAL GROWTH BIOMARKERS | Cátia Lourenço-Marques | S2AQUA |
| 15:00 | PHYTOGENIC STRATEGIES TO ENHANCE GUT RESILIENCE IN SEABREAM | Elena Sarropoulou | HCMR |
| 15:15 | MILD OXIDATIVE STRESS GUIDES DIETARY EPIGENETIC MODULATORS TO DIRECT DNA METHYLATION TOWARD STRESS-RESILIENCE PATHWAYS | Erick Perera | ICMAN-CSIC |
| 15:30 | EPIGENETIC BASIS OF THE ONSET OF SEXUAL SIZE DIMORPHISM IN TELEOSTS | Gabriel Ecker-Eckhofen | ICM-CSIC |
| 15:45 | SINGLE-NUCLEUS TRANSCRIPTOMICS OF ATLANTIC SALMON MUSCLE CAPTURES MYOGENIC CELL STATES AND POST-DUPLICATION DIVERGENCE | Ulduz Sobhiafshar | The Roslin Institute |
| **16:00** | ***Coffee break*** | | |
| **Session 5: Microbial genomics** | | **Chairs: Kiron Viswanath, Dana Silva, Sandra López** | |
| 16:30 | FLAVOBACTERIUM PSYCHROPHILUM GENOMICS TO UNRAVEL EVOLUTION, VIRULENCE AND HOST ADAPTATION OF A MAJOR FISH PATHOGEN | Eric Duchaud | INRAE |
| 16:45 | IDENTIFICATION OF AN ANTIBIOTIC RESISTANCE CLUSTER IN AEROMONAS SALMONICIDA SUBSP. SALMONICIDA ASSOCIATED WITH NEW PLASMIDS AND A-LAYER EXPRESSION | Line Saidan | INRAE |
| 17:00 | PHOTOPERIOD-DRIVEN SEX-SPECIFIC SHIFTS IN BACTERIAL AND FUNGAL GUT COMMUNITIES IN ATLANTIC COD (GADUS MORHUA) | Dana Silva | ICM-CSIC |
| 17:15 | THERMAL AND DEVELOPMENTAL DRIVERS OF MICROBIAL COMMUNITY DYNAMICS IN GILL, INTESTINE AND SKIN OF GILTHEAD SEA BREAM AND EUROPEAN SEABASS DURING EARLY ONTOGENY | Hamed Abdollahpour | CCMAR |
| 17:30 | EFFECTS OF A DIETARY BACTERIOPHAGE TARGETING FLAVOBACTERIUM PSYCHROPHILUM ON THE GUT MICROBIOME OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) | Benjamin Overland | Swansea University |
| 17:45 | PRIMING THE FISH MICROBIOME IN EARLY LIFE TO IMPROVE STRESSOR RESILIENCE | Tamsyn Uren Webster | Swansea University |
| **18:00** | **End of Day 2** | | |

| DAY 3 — WEDNESDAY, 6 MAY 2026 | | | |
|---|---|---|---|
| **Time** | **Title** | **Presenting author** | **Institution** |
| **09:45** | **PLENARY LECTURE — Yann Guiguen (INRAE, France)** | | |
| **~10:30** | ***Coffee break*** | | |
| **Session 6: Integrative omics** | | **Chairs: Paulino Martinez, Arun Shankregowda** | |
| 11:00 | GENOME ARCHITECTURE SHAPES EPIGENETIC AND TRANSCRIPTIONAL DIVERGENCE IN EUROPEAN SEABASS POPULATIONS | Francois Allal | MARBEC Ifremer |
| 11:15 | CELL-TYPE-SPECIFIC ENDOCRINE SIGNALS OF THE PITUITARY GLAND IN RESPONSE TO PHOTOPERIOD MANIPULATION | Ioannis Konstantinidis | Nord University |
| 11:30 | PHOTOPERIOD EFFECT ON ATLANTIC COD GONADS AND PITUITARY REVEALS MODULATION OF GENE EXPRESSION AND METHYLATION | Lorenzo Colonna | Nord University |
| 11:45 | INTEGRATIVE OMICS ANALYSES REVEAL GENOMIC DIFFERENTIATION AND DIVERGENT GENE EXPRESSION ACROSS BROWN TROUT LINEAGES | Manuel Vera | Univ. Santiago de Compostela |
| 12:00 | METHODOLOGICAL INSIGHTS FOR STUDYING LNCRNAS IN FISH: BENCHMARKING TRANSCRIPTOME DEPTH AND EXTRACELLULAR VESICLE RNA PROFILING | Deiene Rodriguez-Barreto | Univ. de La Laguna |
| 12:15 | SHELL-ULAR BIOLOGY: SINGLE NUCLEI-RESOLVED GENE EXPRESSION CHANGES IN THE EPITHELIAL TISSUE OF MOULTING LITOPENAEUS VANNAMEI | Thomas Clark | The Roslin Institute |
| 12:30 | SKIN MUCUS AND TISSUE MULTIOMICS COMPARISON OF TRANSPORT-INDUCED ACUTE STRESS RESPONSES IN ATLANTIC SALMON, EUROPEAN SEABASS AND RAINBOW TROUT | Tonka Buha | CIIMAR ICBAS and SPAROS |
| ***12:45*** | ***Lunch break*** | | |
| **Session 7: Reproduction & improved breeding I** | | **Chairs: Dean Jerry, Renan Appel** | |
| 14:30 | PRECISION GENOMICS IN ARCTIC CHARR: CUSTOM SNP-CHIPS FOR BACTERIAL KIDNEY DISEASE RESISTANCE AND DIVERSITY MANAGEMENT | Christos Palaiokostas | Swedish Univ. of Agricultural Sciences |
| 14:45 | COMPLEXITIES OF SELECTIVE BREEDING WITHIN PEARL OYSTERS – A LONGDITUDINAL STUDY REVEALS MOLECULAR DRIVERS OF SHELL GROWTH AND COLOUR | David Jones | James Cook University |
| 15:00 | THE SPERM RACE MATTERS: PHENOTYPIC AND GENOMIC EVIDENCE FOR WITHIN-EJACULATE HAPLOID SELECTION IN DICENTRARCHUS LABRAX | Francois Allal | MARBEC Ifremer |
| 15:15 | TRANSCRIPTOMIC AND EPIGENETIC CHANGES IN THE OLFACTORY ORGANS OF SENEGALESE SOLE MAY UNDERLIE REPRODUCTIVE FAILURE IN CAPTIVE-BRED MALES | Dorinda Torres-Sabino | Univ. Santiago de Compostela |
| 15:30 | OVULATION DEFINES FINAL MATERNAL RNA LANDSCAPE THROUGH EXTENSIVE TRANSCRIPTOMIC REMODELING IN PIKEPERCH EGGS | Daniel Żarski | InLife Institute |
| 15:45 | TRANSCRIPTOMICS OF RAINBOW TROUT EGGS USED FOR GYNOGENESIS: PROTECTION FROM PHYSICAL STRESS VS. SENSITIVITY OF MICROTUBULES FOR HYDROSTATIC PRESSURE SHOCK | Konrad Ocalewicz | University of Gdansk |
| **16:00** | ***Coffee break*** | | |
| **Session 8: Reproduction & improved breeding II / Low trophic aquaculture** | | **Chairs: Elena Sarropoulou, Ivana Buselic** | |
| 16:30 | ROOTING THE FUTURE OF SPACE AQUACULTURE | Laia Ribas | ICM-CSIC |
| 16:45 | SHARED GENETIC ARCHITECTURE REVEALS PLEIOTROPIC QTL(S) REGULATING GONADOSOMATIC INDEX AND SEXUAL MATURITY IN ATLANTIC COD | Muhammad Luqman Aslam | Nofima |
| 17:00 | REPRODUCTIVE PERFORMANCE OF ZEBRAFISH (DANIO RERIO) CRISPR/CAS9 MUTANT LINES | Tadlo Mengesha | University of Gondar |
| **Session 8: Low trophic aquaculture** | | | |

| | | | |
|---|---|---|---|
| 17:15 | EXPLORING GENETIC DIVERSITY AND HYBRIDISATION PATTERNS OF MUSSELS IN NORTHERN SCOTLAND AND IRELAND | Ambre Chapuis | The Roslin Institute |
| 17:30 | LONG-READ SEQUENCING REVEALS EXTENSIVE STRUCTURAL VARIATION IN FARMED SCOTTISH MYTILUS MUSSELS | Tim Regan | The Roslin Institute |
| 17:45 | FROM CHALLENGE TEST TO GWAS: UNCOVERING GENOMIC SIGNALS OF SALINITY TOLERANCE IN THE COMMON COCKLE | Marialaura Gallo | Univ. Santiago de Compostela |
| **19:00** | **Split Old Town Tour** | | |
| **20:30** | **GALA DINNER** | | |