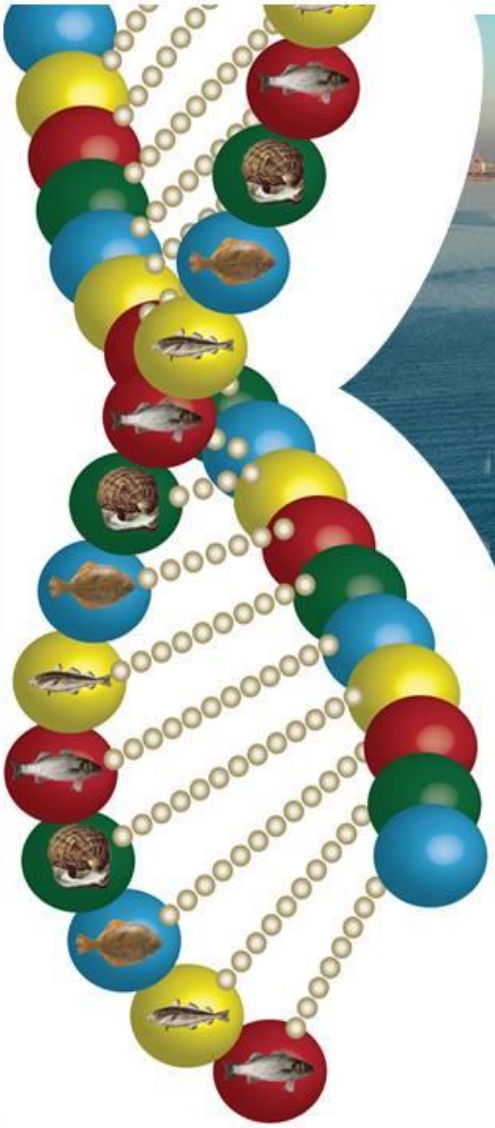
7th International Symposium on Genomics in Aquaculture



GIA2024

Thessaloniki, 22-24 May 2024

Book of Abstracts

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WELCOME

We are extremely delighted to welcome you to Thessaloniki for the 7th edition of Genomics in Aquaculture.

Over a hundred of you have decided to trust GIA again to deliver this conference, where we will have the privilege of enjoying the latest advances in genomics research amongst our colleagues and friends. We want to thank you all, as your support is the sole reason that keeps GIA alive and thriving.

We have worked hard to offer you the best possible programme - we hope you will enjoy the science, social activities and company.

We look forward to meeting you all in Thessaloniki.

GIA 2024 organizing & scientific committees

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INDEX

PROGRAMME

DAY 1

Wed 09:45-13:00	08:30	**REGISTRATION**		
	09:15	**GIA WELCOME**		
		WELCOME SPEECHES Dean of the Faculty of Sciences, Head of the Biology School (Aristotle University of Thessaloniki)		
	09:45	**PLENARY: PROF LUCA BARGELLONI**		
		Coffee break		
		Session 1: Stress and immune response I	**Chairs: Alexandros Triantafyllidis & Roberta Marcoli**	
	11:00	The molecular and cellular repertoires of a teleost tongue – insights into the interplay of gustation and immunity	Carlo Lazado	Nofima
	11:15	Genomic basis of the variation in sea lice susceptibility among Atlantic salmon and coho salmon revealed by single-nuclei RNA sequencing	Sarah Salisbury	The Roslin Institute
	11:30	Use of transcriptomics and gene editing to explore genetic mechanisms giving species-specific host-resistance to sea lice	Nick Robinson	Nofima
	11:45	Immunoglobulin sequencing provides new insight in B cells biology of salmonid fish	Aleksei Krasnov	Nofima
	12:00	Conserved and divergent arms of the antiviral response in the duplicated genomes of salmonid fishes	Thomas Clark	University of Aberdeen
	12:15	Microbiome responses to aquaculture-related husbandry practices	Arun Shankregowda	Swansea University
	12:30	Host-microbiota cross-talk upon gut parasite infection in gilthead seabream	Socorro Toxqui-Rodriguez	IATS-CSIC
	12:45	Chromatin accessibility in gill tissue identifies candidate genes and loci associated with aquaculture relevant traits in tilapia	Tarang Mehta	Earlham Institute
		Lunch break		
Wed 14:30-16:00		**Session 2: Stress and immune response II**	**Chairs: Costas Tsigenopoulos & Lorenzo Colona**	
	14:30	Impact of the incorporation of functional annotation into genomic prediction in a turbot population challenged with *Philasterides dicentrarchi*	Christina Kriaridou	The Roslin Institute
	14:45	Functional genomic architecture of viral nervous necrosis disease resistance in farmed European seabass	Robert Mukiibi	The Roslin Institute
	15:00	Transcriptomic approach to characterize gene expression in mussel (*Mytilus galloprovincialis*) gills and hemocytes from the hemolymph or the intervalvar liquid	Magalí Rey-Campos	IIM-CSIC
	15:15	The genomic basis of the effective immune response of mussels	Amaro Saco	IIM-CSIC
	15:30	Validation of a genomic region associated with bonamiosis status in flat oyster populations at individual level	Inés Sambade	University of Santiago de Compostela

	15:45	Single nuclei RNA-sequencing of WSSV-infected whiteleg shrimp (*Litopenaeus vannamei*) reveals novel genes involved in white spot infection	Alexandra Florea	The Roslin Institute
	16:00	Low metabolism activity and efficient antiviral response enable to counteract the pacific oyster mortality syndrome in non-permissive conditions induced by the temperature	Léo Duperret	Ifremer
	16:15	Signatures of selection for tolerance and resistance to *Perkinsus olseni* in grooved carpet shell clam (*Ruditapes decussatus*) using population genomics	João Estêvão	University of Santiago de Compostela
Wed 16:30-18:30		**POSTER SESSION**		

DAY 2

Thu 09:45-13:00	09:45	**PLENARY: PROF ZE-XIA GAO**		
		Coffee break		
		Session 3: Reproduction and breeding I	**Chairs: Roberto de la Herrán & Elisavet Kaitetzidou**	
	11:00	Applications of genomic selection for complex economic traits in large yellow croaker	Peng Xu	Xiamen University
	11:15	Epigenetic regulation of genetic sex determination of channel catfish explains sex ratio variations with high temperature	John Liu	Tennessee Tech University
	11:30	High-throughput screening of the first chromosome-level genome assembly of *Merluccius merluccius* suggests a sex determining gene underlying a XX/XY system	Paulino Martínez	University of Santiago de Compostela
	11:45	Characterisation of olfactory transcriptome in Senegalese sole (*Solea senegalensis*) at the single-cell level: functional implications in reproduction	Dorinda Torres	University of Santiago de Compostela
	12:00	miRNA phenotypic target identification reveals hippo pathway-mediated regulation of reproduction by miR-202	Julien Bobe	INRAE
	12:15	Genomic regions associated with variable sperm motility across the reproductive season in Artic charr	Khrystyna Kurta	Swedish University of Agricultural Sciences
	12:30	Deciphering the sperm methylation landscape of Swedish Arctic charr with regard to male-fertility traits	Fotis Pappas	Swedish University of Agricultural Sciences
	12:45	Unravelling sex-dependent epigenetics mechanisms in response to environmental cues	Laia Ribas	ICM-CSIC
		Lunch break		
		Session 4: Development and early life interventions	**Chairs: Laia Ribas & Aristotelis Moulistanos**	
Thu 14:30-17:30	14:30	SALMOCODE: A single-cell atlas of Atlantic salmon organogenesis	Christiaan Henkel	Norwegian University of Life Sciences
	14:45	Epigenomics of embryogenesis in the turbot (*Scophthalmus maximus*)	Oscar Aramburu	University of Santiago de Compostela

	15:00	DNA methylation during early development in diploid and triploid European seabass	Francesc Piferrer	ICM-CSIC
	15:15	Integration of multi-omics data to uncover key genes and metabolic pathways involved in flatfish metamorphosis.	Laura Guerrero-Peña	IIM-CSIC
	15:30	Improving Atlantic salmon health and welfare by embryonic temperature programming	Erik Burgerhout	Nofima
	15:45	Correlation between epigenetic and transcriptomic changes in golden barramundi (*Lates calcarifer*): a multiomic approach	Roberta Marcoli	James Cook University
		Coffee break		
		Session 5: Structural genomics and population genetics	**Chairs: Efthimia Antonopoulou & Maria Papadaki**	
	16:30	Broodstock management influences the metabolic status, gene expression and epigenetic gene regulation in progeny	Kaja Skjærven	University of Bergen
	16:45	First insights into genetic differentiation among sympatric morphs of arctic charr based on whole-genome sequencing	Khrystyna Kurta	Swedish University of Agricultural Sciences
	17:00	Unveiling genomic signatures for domestication in European seabass (*Dicentrarchus labrax* L.)	Aristotelis Moulistanos	Aristotle University of Thessaloniki
	17:15	Mitochondrial function is at the frontline of domestication in Eurasian perch (*Perca fluviatilis*)	Daniel Żarski	Polish Academy of Sciences
Thu 19:30-23:30		**GALA DINNER**		

DAY 3

	09:45	**PLENARY: PROF WES WARREN**		
		Coffee break		
		Session 6: Nutrition, growth and pigmentation	**Chairs: Carla Piazzon & Nikolas Panteli**	
	11:00	Transcriptomic adaptation to salinity in Mozambique tilapia takes shorter time than in Nile tilapia	Avner Cnaani	Volcani Institute
	11:15	Candidate list of biological age markers for welfare auditing in farmed gilthead sea bream	Alvaro Belenguer	IATS-CSIC
Fri 09:45-13:00	11:30	A la cart(e) genome-editing for enhanced feeding behavior in Nile tilapia	Jakob Biran	Volcani Institute
	11:45	Early life feeding effects on Nile tilapia gut microbiome and transcriptome	Fotini Kokou	Wageningen University
	12:00	GWAS and accuracy of prediction for growth in meagre *Argyrosomus regius*	Stavroula Oikonomou	HCMR
	12:15	Identification of SNPs and candidate genes associated with growth using GWAS and transcriptome analysis in *Coilia nasus*	Yue Yu	Huazhong Agriculture University
	12:30	Utilizing pool-seq to identify the gene controlling black patches pigmentation in ornamental carp (koi)	Roni Tadmor	The Hebrew University of Jerusalem

	12:45	Genome survey for processes susceptible to manipulation through DNA methylation remodelling in gilthead sea bream	Erick Perera	ICMAN-CSIC

Lunch break

Fri 14:30-21:30	**EXCURSION TO VERGINA**		

POSTER PRESENTATIONS

Title	Presenting author	Institution
P1- WITHDRAWN		
P2- WITHDRAWN		
P3- Deciphering the IGH locus of gilthead seabream	Carla Piazzon	IATS-CSIC
P4- Nutrition and immune function in farmed fish regulation of inflammatory and anti-inflammatory responses	Asma Abushweka	University of Aberdeen
P5- Different sampling approaches for DNA metabarcoding analysis of the stomach contents of the Atlantic bluefin tuna (*Thunnus thynnus* Linnaeus, 1758)	Luka Žuvić	IZOR
P6- Influence of 3'UTR variants on germ cell-specific gene expression in Atlantic salmon	Silja Gulliksen	Mowi Genetics
P7- Mussel antiviral transcriptome response and elimination of viral haemorrhagic septicaemia virus (VHSV)	Amaro Saco	IIM-CSIC
P8- Transcriptomic analysis of turbot (*Scophthalmus maximus*) treated with zymosan a reveals that lncRNAs and inflammation-related genes mediate the protection conferred by β-glucans against *Aeromonas salmonicida*	Alejandro Romero	IIM-CSIC
P9- Genetic structure of native and translocated freshwater crayfish *Pontastacus leptodactylus* populations from Greece and turkey	Maria Alvanou	University of Western Macedonia
P10- Dissecting the genetic basis underlying response against salmonid alphavirus in Atlantic salmon	Domniki Manousi	Norwegian University of Life Sciences
P11- Molecular and physiological responses to chronic stress in European sea bass, *Dicentrarchus labrax*	Athanasios Samaras	University of Crete
P12- Can Atlantic bluefin tuna be efficiently switched from small pelagic fish to pellet feed under farming conditions?	Ivana Buselic	IZOR
P13- Divergent evolutionary paths and ontogenic regulation of proteases in the genome of European sea bass (*Dicentrarchus labrax*)	Rafael Angelakopoulos	University of Thessaly
P14- Serotype variation and genomic characterization of streptococcus agalactiae strains isolated from Nile tilapia	Renan Appel	Nord University
P15- Should we rear tilapia larvae in the dark?	Nikko Cabillon	Hebrew University of Jerusalem
P16- Combined quantitative and population genetics methods provide insight into the genetic landscape of fillet fat in European sea bass *Dicentrarchus labrax*	Stavroula Oikonomou	HCMR
P17- Comparison of tendon tissue differentiation between zebrafish and medaka by single-cell sequencing	Yu Long Chen	Huazhong Agricultural University

P18- Early life programming and molecular regulation of the stress response in greater amberjack (*Seriola dumerili*) larvae	Athanasios Samaras	University of Crete
P19- Genetic analysis of growth traits in fugu, *Takifugu rubripes*	Clémence Fraslin	The Roslin Institute
P20- Evolutionary dynamics of paralogous genes in oxidative phosphorylation and ontogenetic regulation in gilthead seabream (*Sparus aurata*)	Andreas Tsipourlianos	University of Thessaly
P21- Genetic resilience in the common cockle (*Cerastoderma edule*): contrasting natural selection and selective breeding for marteiliosis resistance	Marina Pampin Iglesias	University of Santiago de Compostela
P22- WITHDRAWN		
P23- Applying a chromosome-scale assembly to explore population structure, metagenomics and evolution in the blue mussel (*Mytilus edulis*)	Tim Regan	The Roslin Institute
P24- A heatwave event after hatching alters DNA methylation profile in rainbow trout (*Oncorhynchus mykiss*)	Ignacio Fernández	IEO-CSIC
P25- Due to their improved immunity, disease resistant common carp fish are also less infective	Lior David	The Hebrew University of Jerusalem
P26- A new sex determining gene identified in striped bass	Evgeniya Marcos-Hadad	The Hebrew University of Jerusalem
P27- Genetic analysis and development of sex-specific markers in populations of *Megalobrama amblycephala* based on whole-genome re-sequencing	Lifei Luo	College of Fisheries Huazhong Agricultural University
P28- Gene expression profiling of six6 and bcl6a in larva and juvenile stages in European seabass (*Dicentrarchus labrax* L.)	Aristotelis Moulistanos	Aristotle University of Thessaloniki
P29- Using long-read sequencing with oxford nanopore to enable genomic and epigenomic profiling of livestock fish	Fernando Naya-Catala	IATS-CSIC
P30- The expansion of sirtuin gene family in gilthead sea bream (*Sparus aurata*). Phylogenetic, syntenic and functional insights across the vertebrate/fish lineage	Fernando Naya-Catala	IATS-CSIC
P31- Differential expression of micro RNAs (miRNAs) in gonads of gilthead seabream *Sparus aurata*	Maria Papadaki	HCMR
P32- Molecular identification of bacterial pathogens associated with diseases in aquaculture farms in the Philippines	Casiano Choresca	NFRDI
P33- Understanding the genetic basis of pigmentation anomalies in flatfish	Daniel Costas Imbernón	IIM-CSIC
P34- The effect of dietary *Rhodotorula mucilaginosa* on liver antioxidant gene expression of *Sparus aurata*	Emmanouil Malandrakis	Agricultural University of Athens
P35- GRINNAQUA: green innovation strategies for animal health management: towards sustainable aquaculture	Sergio Fernández-Boo	CIIMAR
P36- WITHDRAWN		
P37- Genetic identification of sturgeon populations from Ukraine using DNA markers	Olha Malysheva	The Leibniz Institute for Zoo and Wildlife Research
P38- Molecular characterization of breeders of *Solea sengalensis*	Laureana Rebordinos	University of Cádiz
P39- A single heatwave event is able to disrupt spermatogenesis in rainbow trout (*Oncorhynchus mykiss*) breeders	Marta Riesco	University of León

P40- A high-quality genome assembly of the Mediterranean mussel (*Mytilus galloprovincialis*) provides insights into mussel sex determination	Raquel Rodríguez-Vázquez	University of Santiago de Compostela
P41- Single-cell transcriptome and epigenetic profiling of the pituitary gland provide insights into teleost puberty	Ioannis Konstantinidis	Nord University
P42- Skmer – genetic diversity metrics from genome skims for broodstock selection	Homère Alves Monteiro	University of Copenhagen
P43- Assessing the impact of insect meal on the intestinal bacterial microbiota of the Baltic shrimp *Palaemon adspersus*	Nikolas Panteli	Aristotle University of Thessaloniki
P44- Study on the developmental characteristics and molecular regulatory mechanisms of different types of intermuscular bone in *Coilia nasus*	Shiming Wan	Huazhong Agricultural University
P45- WITHDRAWN		
P46- Exploring genetic diversity and hybridisation patterns of mussels in northern Scotland	Ambre Chapuis	The Roslin Institute
P47- Broodstock management influences the metabolic status, gene expression and epigenetic gene regulation in progeny	Kaja Skjærven	University of Bergen
P48- Gene expression profile of the cockle *Cerastoderma edule* in response to trematode infection	Simão Correia	CIIMAR
P49- Differential gene expression at different stages of gonadal development in male and female Senegalese sole (*Solea senegalensis*)	Roberto de la Herrán	University of Granada
P50- Genetic diversity of *Plesionika edwardsii*: a shrimp species with economic interest in the Alboran sea region	Francisca Robles	University of Granada
P51- Comparative analysis of gonads from wild and F1 cultivated adult males of Senegalese sole by scRNASeq	Carmelo Ruiz-Rejón	University of Granada
P52- Morphometrics in different genotypic classes of *Dicentrarchus labrax* two developmental stages (Linnaeus, 1758)	Aristotelis Moulistanos	Aristotle University of Thessaloniki

INVITED SPEAKERS

Prof Luca Bargelloni

THE ROLE OF REGULATORY VARIANTS IN NATURAL AND ARTIFICIAL SELECTION: THE CASE OF THE EUROPEAN SEA BASS

University of Padova, Italy

Luca Bargelloni is currently full professor in Animal Biotechnology at the University of Padova, Italy. He has been working in genetics and genomics applied to marine organisms for more than 30 years. He led the genome sequencing of the gilthead seabream, the Myer's icefish, the European sea bass, and the Japanese carpet clam. He follows an approach integrating quantitative genetics and functional genomics, to understand the role of genetic variation in natural population of marine animals as well as in farmed species. He leads a multidisciplinary lab focusing on Genomics, Ecotoxicology, and Microbiology of Marine Animals (GEMMA). Recently, he was responsible for the functional annotation of the European sea bass genome in the EU project Aquafaang and he is coordinating a large EU project on shellfish genetics (ShellFishBoost).

Prof Ze-Xia Gao

DEVELOPMENT MECHANISM AND GENETIC IMPROVEMENT OF FISH INTERMUSCULAR BONES

College of Fisheries, Huazhong Agricultural University, China

Ze-Xia Gao is a Professor of Fish genetics and breeding in College of Fisheries, Huazhong Agricultural University. She is now a recognized leader in the field of fish functional genomics and molecular breeding with the aim to improve the fish economic traits.

Dr. Gao's research involves the use of molecular biology, multiomics analysis, gene-editing technologies to clarify the genetic mechanisms underlying the aquaculture traits. Then precision molecular breeding technologies are applied to enhancing aquaculture to ensure food security. She has systematically conducted research on the development mechanism and genetic improvement of fish intermuscular bones (IBs), which have a significant impact on the consumption, processing, and industrial economic value of freshwater fish in China as well as in the world. She successfully identified the key genes regulating the occurrence and development of IBs, constructed a molecular breeding technology system for improving IBs trait, and successfully bred IBs-free blunt snout bream and grass carp new strains through precise editing of key genes.

Dr. Gao's lab is now carrying out comparative genomic, single-cell transcriptomic analyses and functional assays, to gain novel insights into the origin and variation of IBs in teleost evolution, which will contribute broadly to a conceptual framework in the field of evolutionary biology and heterotopic tendon ossification in humans.

Prof Wes Warren

GENOME-WIDE ADAPTATION IN THE FACE OF EXTREME ECOLOGICAL CHANGE

Department of Animal Sciences, University of Missouri, Columbia, MO, USA

Wesley Warren is a professor of comparative genomics in Department of Animal Sciences, Department of Surgery, Institute for Data Science and Informatics.Dr. Warren is a recognized leader in the field of comparative genomics with a theme of looking at biology with a molecular lens.

His scientific journey started with basic animal physiology and has progressed to single cell transcriptome analysis of a broad spectrum of species. Dr. Warren's research involves the use of whole genome comparative methods to examine genetic adaptation events within existing or newly discovered model organisms as a means to better understand human biology. His research has contributed broadly to a conceptual framework in the field of evolutionary medicine as illustrated by his labs sequencing, assembling and characterizing genomes throughout a broad span of Darwins tree of life. Many of his studies have resulted in highly impactful outcomes benefiting scientific understanding of these species. Today the availability of large volumes of genome sequence data has fundamentally changed our approach to trait discovery, whether it be in the form of genome assemblies, population sequencing, or gene expression variation in bulk or at single cell resolution.

Dr. Warren's lab is now carrying out single nuclei transcriptome experiments to identify a cell types specific molecular profile that contributes to our understanding of a species many phenotypes, that could substantially enlighten the ambiguous path that connects genes to their traits.

ORAL PRESENTATIONS

DAY 1 | Wednesday 22 May

Session 1:

Stress and immune response I

THE MOLECULAR AND CELLULAR REPERTOIRES OF A TELEOST TONGUE – INSIGHTS INTO THE INTERPLAY OF GUSTATION AND IMMUNITY

Lazado, C.C. [1], Iversen, M. [1], Sundaram, A.Y.M. [2], Ytteborg, E. [1] and Burgerhout, E. [1]

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SUMMARY

The tongue is traditionally known as a muscular organ with key functions for taste, mastication, and sensation. It is rich in nerve innervation, blood supply and lymphatic drainage. It is also considered a first-line immune organ in higher vertebrates with specialized macrophages and microbiome populations that influence intestinal immunity. However, our understanding of the interplay of gustation and immunity in lower vertebrates, such as in teleost fish, is limited. Due to its position, the fish tongue is expected to be bombarded with a myriad of stressors and antigens in the aquatic environment and, thus, is expected to mount defence mechanisms. Here, we present the molecular and cellular features of Atlantic salmon tongue, particularly focusing on its immunological functions. Atlantic salmon tongue could mount immune responses to *Yersinia ruckeri ex vivo*, though *in vivo* cohabitation experiment resulted in limited responses. *Y. ruckeri* were localized in the tongue, suggesting they could breach the mucosal barriers. Ongoing works include comparative transcriptomics, both bulk and at a single cell level, microscopy and microbiome analysis, which will be presented at the meeting.

Keywords: Microbiota, Mucosal immunity, Single-cell sequencing, Transcriptomics

GENOMIC BASIS OF THE VARIATION IN SEA LICE SUSCEPTIBILITY AMONG ATLANTIC SALMON AND COHO SALMON REVEALED BY SINGLE-NUCLEI RNA SEQUENCING

Salisbury, S.J. [1], Ruiz Daniels, R. [1], Monaghan, S.J. [2], Bron, J.E. [2], Villamayor, P. [1,3], Gervais, O. [1], Fast, M.D. [4], Sveen, L. [5], Houston, R.D. [6], Robinson, N. [5,7] and Robledo, D. [1]

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SUMMARY

Sea lice parasitism is one of the greatest threats facing the Atlantic salmon (*Salmo salar*) aquaculture industry. However, other salmonids are remarkably immune to this ectoparasite. The skin of Coho Salmon (*Oncorhynchus kisutch*) rapidly swells to engulf attached sea lice, but the mechanisms underlying this effective lice-repellant strategy remain largely unknown. We used single-nuclei RNA sequencing, a technology enabling the gene expression profiling of thousands of individual cells, to investigate the genes and cell types used by Atlantic salmon and coho salmon in response to sea lice. Juvenile fish were exposed to sea lice (*Lepeophtheirus salmonis*) copepodids. Fin and skin samples where lice had attached were taken at 12h, 24h, 36h, 48h, and 60h post exposure and from unexposed fish. Nuclei isolation from each sample was followed by barcoding with a Chromium (10X Genomics), Illumina sequencing, and data analysis with STAR and Seurat. We found both species demonstrated a common wound-healing and immune reaction in response to sea lice. However, the immune response of Atlantic salmon was attenuated, potentially due to greater sea lice immunomodulation of this host species. Coho salmon red blood cells downregulated iron-binding genes, perhaps to discourage iron-seeking lice. Finally, we identified three layers of keratinocytes that each had a unique but complementary role in driving the skin swelling responsible for Coho salmon's sea lice resistance. The genes and pathways we found to underly this resistance could be targeted via gene editing to improve Atlantic salmon's innate immunity to this parasite.

Keywords: snRNAseq, salmon, sea lice, cell type, disease resistance

USE OF TRANSCRIPTOMICS AND GENE EDITING TO EXPLORE GENETIC MECHANISMS GIVING SPECIES-SPECIFIC HOST-RESISTANCE TO SEA LICE

Robinson, N.A. [1,2], Østbye, T-K.K. [1], Salisbury, S. [3], Sveen, L. [1], Fast, M. [4], Krasnov, A. [1], Daniels, R.R. [3], Tengs, T. [1], Edvardsen, R.B. [5], Kjærner-Semb, E. [5], Bizuayehu, T.T. [5], Monaghan, S.J. [6], Øvergård, A-C. [7], Doherty Midtbø, H.M. [7], Bron, J.E. [6], Wargelius, A. [5], Baranski, M. [8], Houston, R. [9] and Robledo, D. [3,10]

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SUMMARY

The infestation and removal of sea lice is an intractable problem affecting Atlantic salmon welfare and aquaculture. No existing lice control measures are completely effective. However, genetic variation in host resistance to sea lice exists both within populations of Atlantic salmon and between different species of salmonids, with some species, such as coho, able to mount an effective immune response. Single nuclei RNAseq and spatial transcriptomics has been used to highlight host genes that are differentially expressed in specific cell types at the area of louse attachment after infection in Atlantic and coho salmon. Here we devise and apply a set of criteria to help generate a prioritised list of genes to test further using CRISPR-Cas9 gene editing. Guide RNAs for gene editing have been designed, and eggs injected, to knockout the transcription of specific genes in Atlantic, pink and coho salmon. The exploration of different strategies for knocking-in and upregulating genes in Atlantic salmon will also be discussed. Edited fish and controls will be challenged with sea lice to test whether disruption of the expression of these genes affects attached lice numbers, proliferation/infiltration of granulocytes around the lice attachment site and encapsulation/mortality of sessile sea lice. In this way we will test if specific genes have a functional role giving host-resistance to sea lice in some salmon species.

Keywords: Sea lice, snRNAseq, spatial transcriptomics, CRISPR-Cas9, salmon host resistance

IMMUNOGLOBULIN SEQUENCING PROVIDES NEW INSIGHT IN B CELLS BIOLOGY OF SALMONID FISH

Krasnov, A. [1], Karlsen C. [1], Lund, H. [2], Bakke, A. [2], Bøysen, P.[2], Jimenez-Gurrero R.[2], Mørkøre T.[2], Boudinot, P.[3] and Afanasyev, S.[4.]

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SUMMARY

The variable region of immunoglobulin heavy chain is produced by somatic recombination of V, D and J genes with insertions and deletions at junctions, creating a hypervariable region also known as CDR3. We developed parallel sequencing to assess the size and complexity of IgM repertoire and to detect antibodies responses to vaccination and infections with pathogens. Most innovative findings were related to traffic of responding B cells, which can be an important and previously unknown immune mechanism in salmonids. Each unique VH sequence (*ie*, clonotype) can serve as a barcode of a B cell clone. Detection of identical clonotypes in different tissues or locations of the same fish indicates migration of B cells. Combination of sequencing with analyses of developmental markers can therefore elucidate B cells differentiation, proliferation, and migration. We thus observed enhanced recruitment of B cells from spleen to heart in fish infected with salmon alphavirus (Bakke et al., 2021), suggesting that targeted delivery of antibodies to the infected sites may compensate for their low affinity. Another study focusing on melanized foci found antigen-stimulated differentiation of naïve B cells, which was followed by massive migration from muscle foci towards other tissues (Jimenez-Guerrero et al., 2023), suggesting that this local B cell response could develop into systemic immunization. In this context, pronounced stimulation of B cells traffic during smoltification (Lund et al., 2022) may be regarded as preadaptation to a new environment.

Keywords: Atlantic salmon, B cell, IgM, sequencing

CONSERVED AND DIVERGENT ARMS OF THE ANTIVIRAL RESPONSE IN THE DUPLICATED GENOMES OF SALMONID FISHES

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SUMMARY

Antiviral innate immunity is orchestrated by the interferon system, which appeared in ancestors of jawed vertebrates. Interferon upregulation induces hundreds of interferon- stimulated-genes (ISGs) with effector or regulatory functions. Here we investigated the evolutionary diversification of ISG responses through comparison of two salmonid fishes, accounting for the impact of sequential whole genome duplications ancestral to teleosts and salmonids. By analysing the transcriptomic response of the IFN pathway in the head kidney of both rainbow trout and Atlantic salmon in parallel *in vitro* and *in vivo* experiments, we identified a large set of ISGs conserved in both species and cross-referenced them with zebrafish and human ISGs. Around one-third of salmonid ISG lacked orthologs in human, mouse, chicken or frog, and often between rainbow trout and Atlantic salmon, revealing a fast-evolving, lineage-specific arm of the antiviral response. This study also provides a key resource for in-depth functional analysis of ISGs in salmonid species of commercial relevance.

Keywords: Aquaculture, Comparative genomics, Anti-viral, Immune-response, IFN, Salmonids

Acknowledgements: The AQUA-FAANG project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement 817923.

MICROBIOME RESPONSES TO AQUACULTURE-RELATED HUSBANDRY PRACTICES

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SUMMARY

Routine aquaculture practices, particularly those applied at early life stages, are stressful for fish and can have lasting effects on their health by compromising their immune system. While this is broadly known, little attention has been paid to the consequences of common husbandry practices (e.g., handling, confinement, disinfection) on the composition and structure of the fish microbiome, which plays a critical role on nutrient absorption and development of the fish immune system. We have analysed the effect of common husbandry practices (air exposure, crowding, disinfection, forced hatching, diet changes) on the microbiome composition of food-related aquaculture species (Atlatic salmon, tilapia, rainbow trout) and on species cultured for research and ornamental purposes (zebrafish and killifish). Our results indicate that these common practices are associated with pronounced changes in the fish gut and skin microbiota, resulting in a decline of taxa commonly associated with healthy fish (e.g., Lactobacillales) and the increase of others which include oportunistic pathogens (e.g., Vibrio or Aeromonas). Some of these changes persist over time and indicate that microbiome disruption can be playing an important role on the negative effects associated to routine, yet stressful, aquaculture operations.

Keywords: Stress, Microbiome, Atlantic salmon, Rainbow trout, Research and ornamental fish

HOST-MICROBIOTA CROSS-TALK UPON GUT PARASITE INFECTION IN GILTHEAD SEABREAM

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SUMMARY

The enteric myxozoan parasite *Enteromyxum leei* is an important problem in gilthead seabream aquaculture. The parasite invades the intestinal epithelium causing chronic intestinal inflammation, poor food conversion rates, cachexia, and mortalities, resulting in significant economic losses. Gut microbiota has key effects on host health and welfare, including disease resistance and immune function, tightly interacting with the host affecting systemic and local physiological functions. The aim of this study was to gain insights into the host-microbiota-parasite interactions integrating transcriptomics, metataxonomics, and metatranscriptomics within this disease model. Intestinal tissue and mucus were obtained from seabream after 10 weeks of parasite exposure. Microbial and host transcripts were sequenced by Illumina, whereas metataxonomics was studied using ONT MinION sequencing. Unexposed animals were used as a control. Parasite exposure significantly changed gut microbiota, with a decrease in diversity and an increase in the Moraxellaceae family, and altered the expression of 936 host genes, highlighting genes involved in interferon and interleukin signaling, cellular response to hypoxia and cellular stress and apoptosis. Microbial transcripts, including bacterial, fungal, and viral transcripts, also showed significant changes upon parasite infection. Integration of the results revealed differential effects on the host induced directly by the parasite (e.g., Toll-like receptor signaling) or indirectly by the parasite-induced microbial shift (e.g., Cytokine signaling). These results provided different sets of bacterial taxa and microbial and host transcripts that allow a better understanding of this host-microbiota-parasite interaction and can serve as biomarkers for evaluating mucosal health in aquaculture.

Keywords: *Sparus aurata*, intestinal parasites, microbiota, transcriptomics, metatranscriptomics

CHROMATIN ACCESSIBILITY IN GILL TISSUE IDENTIFIES CANDIDATE GENES AND LOCI ASSOCIATED WITH AQUACULTURE RELEVANT TRAITS IN TILAPIA

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SUMMARY

The Nile tilapia (*Oreochromis niloticus*) accounts for ~9% of global freshwater finfish production. The exponential growth in tilapia aquaculture production is largely due to their suitability for aquaculture systems, with the ability to grow and reproduce in many culture systems. However, climate change is leading to extreme cold events and competition is decreasing freshwater resources. Therefore, there is a need to develop aquaculture systems based on saline waters with broad temperature ranges. By determining the genetic bases responsible for such adaptive traits e.g. salinity acclimation, we can genotype and breed desirable traits into farmed strains.

We recently reported striking cases of gene regulatory network (GRN) rewiring for adaptive trait genes, confirming that discrete transcription factor binding site (TFBS) mutations disrupt regulatory edges in the *O. niloticus* genome. To further genotype the observed variation at *O. niloticus* gene regulatory sites and assess the impact on gene expression, we generated ATAC-seq and RNA-seq data from *O. niloticus* gill tissues. Through the integration of SNPs from 27 tilapia species, identified 1,168 highly expressed genes (4% of all Nile tilapia genes) with highly accessible promoter regions with functional variation at transcription factor binding sites (TFBSs). Regulatory variation at these TFBSs is likely driving gene expression differences associated with tilapia gill adaptations, and differentially segregate in freshwater and euryhaline tilapia species. The generation of novel integrative data revealed candidate genes e.g., *prolactin receptor 1* and *claudin-h*, genetic relationships, and loci associated with aquaculture relevant traits like salinity and osmotic stress acclimation.

Keywords: Aquaculture, Epigenetics, Tilapia, Regulatory evolution, Molecular evolution

Session 2:

Stress and immune response II

IMPACT OF THE INCORPORATION OF FUNCTIONAL ANNOTATION INTO GENOMIC PREDICTION IN A TURBOT POPULATION CHALLENGED WITH *PHILASTERIDES DICENTRARCHI*

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SUMMARY

Infectious diseases represent a major threat to farmed animals, having a large impact on animal health, welfare, production, and compromising human food security. Genetic disease control strategies focusing on the hosts response to pathogens have gained attention in recent years. Disease resilience, the ability of an animal to cope and survive an infectious disease, has emerged as a desirable breeding goal. In this study, we used whole-genome sequencing (WGS) to genotype SNPs and structural variants (SVs) in a population challenged with the parasite *Philasterides dicentrarchi*, a ciliate that causes scuticociliatosis resulting in high mortality in farmed flatfish. The primary objectives were to assess if WGS combined with functional annotation might i) produce an accurate picture of the genetic architecture of disease resilience traits and ii) increase the accuracy of selection. The 54 parents of the challenged families were whole-genome sequenced, and their genotypes served as reference to impute the SNPs and SVs of their offspring. The resulting dataset was used to estimate the genetic parameters of resilience traits (days-to-death, infectivity, susceptibility and recoverability, estimated using an epidemiological model), and conduct a genome-wide association study (GWAS), which revealed a polygenic genetic architecture for all traits. The genome's functional annotation, obtained from the AQUA-FAANG project, was incorporated to test the accuracy of genomic prediction using BayesRCO. Overall, GBLUP consistently outperformed Bayesian models except for the “days-to-death” trait, for which the Bayesian performed similarly to GBLUP. In this dataset, the incorporation of functional annotation did not improve predictions of the tested traits.

Keywords: turbot, functional annotation, disease resistance, selective breeding

FUNCTIONAL GENOMIC ARCHITECTURE OF VIRAL NERVOUS NECROSIS DISEASE RESISTANCE IN FARMED EUROPEAN SEABASS

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SUMMARY

Viral nervous necrosis (VNN), caused by the nervous necrosis virus (NNV), is a major infectious disease threatening the European seabass aquaculture industry. VNN causes high economic losses emanating from high mortality rates and slow growth of infected fish. Selective breeding has the potential to increase the resistance of European seabass to VNN, and recent studies have identified major QTLs associated with this trait, however, the genes and genomic variants modulating resistance to the disease remain unknown. In the current study, we integrated multiple functional genomic tools including sequence-based GWAS, eQTL, ATAC- seq, and ChIP-seq to identify genes and causal variants modulating VNN resistance in farmed European seabass. As in the previous studies, our results demonstrated that VNN resistance is moderately heritable ($h^2 = 0.45$). Additionally, GWAS results confirmed a major VNN resistance QTL on Chromosome 3. Variants in this region explained up to 38.3% of the additive genetic variance, and the most significant were located within the *IFI27L2A* gene. Furthermore, our analyses showed a remarkable association between *IFI27L2A* gene expression and VNN resistance in the brain and head kidney, and an eQTL analysis revealed that the major QTL variants also had a significant impact on the expression of *IFI27L2A* in both tissues. Finally, ATAC-seq and ChIP-seq analyses identified three QTL/eQTL SNPs overlapping with active chromatin regions putatively regulating the expression of the *IFI27L2A* gene. *G*enetic analyses across wild seabass populations with different levels of resistance to VNN pinpoint one of the variants as the causative mutation underlying the VNN resistance QTL.

Keywords: VNN, European seabass, GWAS, eQTL, ATAC-seq

TRANSCRIPTOMIC APPROACH TO CHARACTERIZE GENE EXPRESSION IN MUSSEL (*Mytilus galloprovincialis*) GILLS AND HEMOCYTES FROM THE HEMOLYMPH OR THE INTERVALVAR LIQUID

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SUMMARY

Hemocyte cells represent the main component of the internal self-defense system of mussels (*Mytilus galloprovincialis)* and they are usually studied to analyze these animals' immune response. However, hemocytes are not only present in hemolymph since they have been detected outside the mussle's body, in the intervalvar liquid, constituted essentially by sea water.

Intervalvar cells show marked differences in comparison to hemolymph cells. Intervalvar cells are smaller than those of the hemolymph, their number is significant higher during a bacterial infection or tissue injury and those external cells move more and faster than hemolymph cells. In terms of immune functions, ROS production is significant higher in intervalvar cells compared to hemolymph cells.

All the aforementioned characteristics pointed to a marked functional differentiation between the two cell groups. A RNA-seq has been performed in order to complete the characterization of this new cell group and compare their gene expression to the other two key players in the mussel immune response: hemolymph cells and gills. Transcriptomics confirmed the highly specialization of the three sample types under study. Gills showed a significant modulation of genes related to cellular movement and calcium metabolism. Intervalvar cells showed an "activated state" compared to the hemolymph cells, being significantly over-expressed apoptotic genes, oxidative genes and HSP70. Finally, a *Vibrio splendidus* waterborne infection showed again the specialization of the different cell groups and gills.

The results indicate that intervalvar cells could constitute the first line of defense as external sentinels extending the immunological alert system outside of the mussel body.

Keywords: mussel, intervalvar cells, immune response, transcriptomics.

THE GENOMIC BASIS OF THE EFFECTIVE IMMUNE RESPONSE OF MUSSELS

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SUMMARY

Mussels (*Mytilus galloprovincialis*) are characterized by a great capacity of adaptation and a very effective innate immune system. They are filter feeders but they are capable of surviving without major mortalities in the pathogen-rich marine environment. The sequencing of the mussel genome and of several resequenced individuals in 2020, revealed the first open pangenome in a metazoan, with a large fraction of hemizygous genes being subjected to presence/absence variation (PAV) between individuals (30% of the mussel gene set). Clear differences were observed between the dispensable repertoires of resequenced individuals from two populations. The set of dispensable genes was enriched significantly in immune gene families. Several immune gene families present impressive expansions in this species, including lectins, C1qs, Toll-like receptors (TLR) or interleukins-17. The mussel TLR repertoire is the most expanded and diverse one in all Metazoa, and functional diversity was described as the leading diversifying force. All of these immune gene families are affected by PAV in some degree. In some of them, like myticins, a mussel specific gene family with impressive expression levels, PAV is so strong that affects subfamilies. The information presented points towards a clear implication of the mussel genomic characteristics in their adaptation capacity and in the efficiency of their immune response. Further research should focus in studying if these phenomena occur in other bivalve species as well.

Keywords: Mussel, bivalves, pangenome, innate immunity, evolution

VALIDATION OF A GENOMIC REGION ASSOCIATED WITH BONAMIOSIS STATUS IN FLAT OYSTER POPULATIONS AT INDIVIDUAL LEVEL

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SUMMARY

European flat oyster (*Ostrea edulis*) is an ecologically and economically appreciated marine bivalve whose populations have been affected by overexploitation of natural beds, habitat degradation, introduction of non-native species and epidemic outbreaks such as *Bonamia ostreae*. The main objectives of this study were: i) to validate at individual level a set of single nucleotide polymorphisms (SNPs) previously associated with the bonamiosis status of populations; and ii) to confirm and establish a fine mapping of the putative inversion on chromosome 8, where those SNPs are located. A sample of 300 oysters from three different Irish shellfish beds including: i) a naïve population, ii) a mid-term affected population (10 years), and iii) long-term affected population (several decades), were studied. Heart and gill samples were collected for estimating parasite load and SNP genotyping, respectively. A multispecies SNP chip comprising ~ 5000 SNPs and including the 20 SNPs panel associated with bonamiosis-resilience was used for genotyping using an Axyom platform. qPCR on a rDNA amplicon was used to estimate parasite load on each sample. Association between genotyping and parasite load is currently being investigated at individual and population level considering their bonamiosis status. Furthermore, long-read sequencing is being carried out on resilient and susceptible oysters to confirm and establish the boundaries of a suggested inversion on chromosome 8 spanning ~29 Mb and to develop a straightforward PCR tool for its genotyping. We expect that the information obtained will aid for marker assisted selection programs for obtaining bonamiosis resilient strains to recover flat oyster populations.

Keywords: *Ostrea edulis*, Single Nucleotide Polymorphism, *Bonamia ostreae*, chromosome inversion

SINGLE NUCLEI RNA-SEQUENCING OF WSSV-INFECTED WHITELEG SHRIMP (LITOPENAEUS VANNAMEI) REVEALS NOVEL GENES INVOLVED IN WHITE SPOT INFECTION

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SUMMARY

White spot syndrome virus (WSSV) is one of the most significant threat to whiteleg shrimp (*Litopenaeus vannamei*) aquaculture production globally, causing significant economic losses due to its rapid spread and high mortality rate. Efforts to limit the impact of this disease are impeded by a lack of effective treatments and the absence of an adaptive immune system in crustaceans. In this context, genome editing represents a promising strategy to develop WSSV-resistant strains that ensure the productivity and sustainability of the whiteleg shrimp industry. However, genome editing requires prior knowledge to prioritize a set of target loci.

Here, we have used single-nuclei RNA sequencing of WSSV-infected and non-infected shrimp to identify genes involved in the WSSV infection process. To achieve this, we optimized disease challenge protocols in adult shrimps, developed new protocols for nuclei extraction and processing of the hepatopancreas and lymphoid organ, and compiled cell atlases of these two tissues to increase our understanding of the host transcriptome. Differential analyses between infected and control animals revealed key cell types and genes involved in the response to WSSV in whiteleg shrimp. The candidate genes resulting from this study will serve as a foundation for future CRISPR-cas9 gene editing studies using *in vitro* models in the first instance, setting a baseline for developing therapeutic strategies to combat WSSV in shrimp aquaculture.

Keywords: Transcriptomics, shrimp, whitespot, nuclei, RNA-seq

LOW METABOLISM ACTIVITY AND EFFICIENT ANTIVIRAL RESPONSE ENABLE TO COUNTERACT THE PACIFIC OYSTER MORTALITY SYNDROME IN NON-PERMISSIVE CONDITIONS INDUCED BY THE TEMPERATURE

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SUMMARY

Crassostrea gigas is the main oyster species produced worldwide for the food industry. Introduced for its high adaptability to different environments, its production has been impacted since 2008 in France by the panzootic disease Pacific Oyster Mortality Syndrome (POMS). Responsible for yearly high mortality rates (up to 100% of the animals), the worsening of this disease was due to the emergence of a particular variant of the *Ostreid Herpesvirus* thus called OsHV-1 µVar. While the polymicrobial nature of POMS has been documented, the underlying mechanisms of the numerous factors that influence this disease remain to be characterized. Seawater temperature is one of those since mortality in the field only occurs between 16°C and 24°C. To comprehend its influence on the disease, two POMS' susceptible full-sib families were generated. They were acclimatized for 16 days at 13, 17, 23 and 30°C and exposed to the disease through a cohabitation design with infected individuals. Oysters under permissive conditions (17 and 23°C) displayed a higher mortality rate than those in non-permissive conditions (13 and 30°C). A multi-omics approach (RNA-seq, metabolomic and metabarcoding) was used to understand the mechanisms underlying the contrasted phenotypes obtained. While disease development was maximum in permissive conditions supported by a high metabolism activity, we show that animals acclimatized in the non-permissive conditions display a more efficient antiviral response. Altogether, our results show that metabolism and antiviral immune response explain the contrasted phenotypes observed under different temperatures during a POMS infection.

Keywords: multi-omics, antiviral, metabolism, disease, *Crassostrea gigas*

SIGNATURES OF SELECTION FOR TOLERANCE AND RESISTANCE TO *PERKINSUS OLSENI* IN GROOVED CARPET SHELL CLAM (*RUDITAPES DECUSSATUS*) USING POPULATION GENOMICS

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SUMMARY

Ruditapes decussatus is a commercially important bivalve species declining by massive mortalities associated with biotic and abiotic factors. *Perkinsus olseni* infections represents a major threat for the species and its control has relied on a combination of management strategies. Understanding the genetic basis of tolerance/resistance to perkinsosis can aid genetic breeding programs for obtaining more resilient strains. We aimed to identify genetic markers associated with perkinsosis resilience in *R. decussatus* using a population genomics strategy. Our hypothesis is that common genomic regions associated with perkinsosis resilience could be disclosed taking as reference the neutral background of *R. decussatus* across the NE Atlantic (NE-A) and Mediterranean (Med) areas. Six beds (including five heavily affected and one naïve) distributed across NE-A (Noia, Pontevedra, Algarve) and Med (Sardinia, Venice, Izmir) were sampled (~30 clams/bed) recording the parasite infection individually. Genetic structure was ascertained using 2b-RADseq genotyping (13,438 SNPs) taking a new *R. decussatus* draft genome as reference. Outlier loci were identified considering both the different degree of perkinsosis infection (tolerance) and the presence/absence of the parasite (resistance) across affected beds. Genetic diversity was lower in the NE-A than in the Med beds (He: 0.168 vs 0.210) and two main clusters were identified separating NE-A and Med areas. Venice plotted between the two main clusters, suggesting restocking with seed from both origins. We identified 84 and 10 outliers of divergent selection associated with tolerance and resistance, respectively, many of them correlated with the level of infection, suggesting their potential for breeding programs.

Keywords: *Ruditapes decussatus*, SNP genotyping, Population genomics, *Perkinsus olseni*, Disease tolerance

DAY 2 | Thursday 23 May

Session 3:

Reproduction and breeding I

APPLICATIONS OF GENOMIC SELECTION FOR COMPLEX ECONOMIC TRAITS IN LARGE YELLOW CROAKER

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SUMMARY

The large yellow croaker is one of the most important mariculture species, with the highest annual mariculture production in China. With the fast development of large yellow croaker culturing, this industry is confronting a series of problems, such as frequent disease outbreaks, disruption of the marine ecological system caused by the consumption of forage fishes, and germplasm degradation. To overcome these bottlenecks, we constructed numerous genetic tools, including chromosome-level genome sequences, genome-wide SNPs, high-throughput SNP arrays, genomic selection models, etc. Using these genetic tools, we conducted genomic selection for resistance against *Cryptocaryon irritans* and *Pseudomonas plecoglossicida*, swimming performance, and highly efficient utilization of plant protein. The heritability of these traits was calculated, and genome-wide association analysis revealed that these traits were all complex quantitative traits controlled by polygenes. The results of infection challenges in the F3 generation showed that the survival rate of the strain resistant to *Cryptocaryon irritans* was 40.8% higher than that of the unselected line. For the strain resistant to *Pseudomonas plecoglossicida*, we bred it to the F2 generation, and the offspring evaluation showed an 18.6% higher survival rate compared to the unselected line. The swimming performance of the offspring from the selected line was 15.8% higher than that of the non-selected line. The growth results of the F2 generation of the selected line with highly efficient utilization of plant protein showed a 16.3% higher weight gain rate compared to the non-selected line. In the future, genetic improvement based on a multi-trait selection index of disease resistance and robustness traits will be the focus of genomic selection breeding for large yellow croakers.

Keywords: Genomic selection, Phenotypic measurement, Genotype, Disease resistance

EPIGENETIC REGULATION OF GENETIC SEX DETERMINATION OF CHANNEL CATFISH EXPLAINS SEX RATIO VARIATIONS WITH HIGH TEMPERATURE

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SUMMARY

Sex of channel catfish is determined by an X/Y system. However, high temperature leads to sex reversal of genetic males into phenotypic females. In this work, epigenetic profiles of DNA methylation were determined from genetic males and females. Hypermethylation of the X alleles was found that led to silencing of the X-borne hydin gene, whereas hypomethylation of the Y allele was found. This would mean expression of hydin gene in genetic males, but not in genetic females. Treatment with a methylation inhibitor, 5-aza- 2'-deoxycytidine, reduced the level of genome-wide DNA methylation, erased the epigenetic marks between genetic males and females within the SDR, and caused masculinization of channel catfish. Accordingly, after the treatment of the methylation inhibitor, hydin-L1, along with six other genes were upregulated, and three genes related to female sex differentiation, esrrg, pard6a, and actrt3, were down-regulated in genetic females. This work demonstrated that the mechanism of genetic sex determination in channel catfish is regulated by epigenetic modification through differential DNA methylation. In such a system, chromosomal sex determination provides a foundation for the sex potential to be male or female, but the final expression and balance of genes for proliferative growth and differentiation determines the outcome of sex. Such epigenetic control of sex determination provides insights into mechanisms of sex determination of lower vertebrates, the linkage of genetic sex determination and temperature sex determination and other environment sex determination and sex differentiation, and explains sex reversal of genetic males into phenotypic females under high temperature with channel catfish.

Keywords: Sex determination, Catfish, DNA methylation, Epigenetic regulation

HIGH-THROUGHPUT SCREENING OF THE FIRST CHROMOSOME-LEVEL GENOME ASSEMBLY OF *MERLUCCIUS MERLUCCIUS* SUGGESTS A SEX DETERMINING GENE UNDERLYING A XX/XY SYSTEM

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SUMMARY

European hake (*Merluccius merluccius*) is a species of great commercial value distributed throughout European coasts. Because hake displays important sex growth dimorphism, a cost-effective molecular tool for sexing would aid for estimating deviations on sex ratio in a scenario of climatic change and strong size- selective fishing mortality. We present the first chromosome-level genome of a European hake female assembled from both long- and short-read sequencing, and further scaffolded using Hi-C. The genome was assembled in 62 scaffolds (32 Mb N50) that were placed into 21 chromosomes spanning 715 Mb. We performed RNA-Seq on muscle, liver, brain, spleen and gonad from pools of individuals for improving annotation (26,625 genes and 41,543 isoforms). We re-sequenced five males and five females using 30X coverage 150 bp PE Illumina sequencing to look for sex-associated markers and to identify the SD gene. Around 12M SNPs were consistently called and, after filtering by MAF>0.3, read depth between 10 and 300 and no missing data in all the 10 individuals, a total of 1,552,561 SNPs were retained for genome screening. We identified two candidate regions on chromosome 9 associated with sex: (i) a stretch of 7 kb where most SNPs were heterozygous in males and homozygous in females close to a member of the sox gene family, and (ii) a large region (several kb) present in females but absent in males, both compatible with a XX/XY system. These markers are being validated in a large sample of males and females using Mass ARRAY.

Keywords: Sex determination, European hake, whole genome sequencing, XX/XY system

CHARACTERISATION OF OLFACTORY TRANSCRIPTOME IN SENEGALESE SOLE (*Solea senegalensis*) AT THE SINGLE-CELL LEVEL: FUNCTIONAL IMPLICATIONS IN REPRODUCTION

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SUMMARY

The reproduction in captivity of the emerging aquaculture species *Solea senegalensis* still faces challenges, notably the presence of a behavioural reproductive dysfunction in offspring males born in farms (F1). Olfaction is essential for chemical communication in the sea-bottom where flatfish live and it has been hypothesized to underlie sole courtship failure, environmental factors associated with different life- stories (F1 *vs* wild) potentially impacting F1 males' performance. Accordingly, we characterized the primary fish olfactory receptors described (OLFC, OR, ORA, TAAR) in Senegalese sole olfactory organs by combining: i) an orthology analysis with well- annotated teleost genomes, ii) a consistent olfactory transcriptome using Nanopore long-read sequencing from a pool of individuals (juveniles, adults, males, females), and iii) single-nuclei short-read RNA-sequencing of wild (2 male, 1 female) and F1 (2 male, 1 female) individuals. Long and short reads were aligned against the reference transcriptome with minimap and analyzed with StringTie for transcriptome characterization, including isoform identification. We identified 18,848 genes exhibiting 49,948 isoforms (FPKM>1), with only 11,360 genes and 13,827 isoforms registered in Ensembl. Among the 311 olfactory receptor genes identified based on orthology, 187 were present in our transcriptome, 161 genes and 339 isoforms exhibiting FPKM>1. Single-nuclei RNA-seq data is currently being explored to assess the cellular context and potential function of these receptors. Sole's olfactory system is functionally active, exhibiting numerous genes and transcripts variations among individuals and conditions, warranting further exploration by analysing the Illumina data with STARsolo and Seurat, to characterize the olfactory organ at single-cell level.

Keywords: Senegalese sole, long and short read, olfactory receptors, transcriptome, single-nuclei RNA-seq.

MiRNA PHENOTYPIC TARGET IDENTIFICATION REVEALS HIPPO PATHWAY-MEDIATED REGULATION OF REPRODUCTION BY MIR-202

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SUMMARY

MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression involved in the regulation of many physiological processes. Functional studies in vertebrates, including fish, have shown that miRNAs can significantly contribute to phenotypic outcomes. In various aquaculture fish species, transcriptomic analyses resulted in the identification of miRNAs preferentially expressed in male and female gonads. Among them, miR-202 exhibited a remarkable gonad-predominant expression, that appears to be evolutionarily-conserved. In the ovary, miR-202 is dynamically expressed throughout the reproductive cycle. These results strongly suggest a major role for miR-202 in fish reproduction. To functionally strengthen this hypothesis, we used a model fish species to knock-out the mir-202 gene. The mir-202 KO resulted in a major reproductive phenotype characterized by lower fecundity and reduced fertilization. Currently, a major limitation of miRNA research is the lack of a suitable strategy to identify phenotypic targets (i.e., target whose de-repression contributes to the phenotypic outcomes) among a high number of computationally-predicted putative targets. Using ovarian RNA-seq data, we used specific criteria including limited fold-change and low interindividual variability in gene expression to reduce the list of 2,853 computationally-predicted putative targets to a short list of 5. We selected tead3b, a member of the evolutionarily-conserved Hippo pathway, due to its remarkably strong and evolutionarily-conserved binding affinity for miR-202-5p. Deleting the miR-202-5p binding site in the 3' UTR of tead3b triggered a reduced fecundity phenotype. This is one of the few successful examples of *de novo* functional assignment of a miRNA phenotypic target *in vivo* in vertebrates.

Keywords: RNA-seq, ovary, miRNA, egg, fecundity

GENOMIC REGIONS ASSOCIATED WITH VARIABLE SPERM MOTILITY ACROSS THE REPRODUCTIVE SEASON IN ARTCIC CHARR

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SUMMARY

This is the first report aiming to highlight genomic regions associated with changes in Arctic charr sperm quality over the reproductive season. Animals with natural and delayed spawning were sampled three times for two consecutive years (2021 and 2022), and a total progressive sperm motility (PM) was recorded using a computer-assisted sperm analysis (CASA). Whole-genome sequencing (WGS) and double digest restriction-site associated DNA sequencing (ddRAD-seq) were applied on males with consistently high (n = 8 WGS; n = 63 ddRAD-seq) versus variable PM (n = 8 WGS; n = 31 ddRAD-seq) across the reproductive season.

During 2021 sampling, 29% of males with natural spawning and 42% of males with delayed spawning had a highly variable PM. During 2022 sampling, 56% of males with the natural spawning showed a high variability PM across the season. Males sampled in 2021 showed significantly higher sperm motility during the early and middle spawning season compared to the same males in the following year. FST scan (FST >0.17) detected genomic differentiation among males with high and low variability in PM on eight (WGS) or 12 chromosomes (ddRAD). Overlapping regions between the WGS and ddRAD datasets were observed on three chromosomes LG11, LG15, and LG18. Genome-wide windows with the highest FST (0.2 - 0.4) on each chromosome contained SNPs within 250 kb-proximity to 16 genes with sperm quality biological functions in mammalian species. The new insights can be valuable to enhance reproductive performance in selective breeding where sperm from single males is used to fertilize individual egg batches.

Keywords: Arctic charr, sperm motility, spawning season, whole-genome sequencing, ddRAD-seq

DECIPHERING THE SPERM METHYLATION LANDSCAPE OF SWEDISH ARCTIC CHARR WITH REGARD TO MALE-FERTILITY TRAITS

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SUMMARY

Reproductive success of captive-born aquaculture species is substantially lower compared to their wild counterparts. Arctic charr (*Salvelinus alpinus*) is a salmonid species of significant value for Nordic aquaculture, but low reproductive success rates are a major issue. A substantial amount of literature suggests that epigenetic factors influence fertility in a wide range of animal species. In this study, the link between sperm DNA methylation patterns and sperm quality characteristics of Arctic charr was evaluated. Semen from male charrs (n=47) was phenotyped for sperm density and kinematic parameters using cytometry and computer-assisted sperm analysis. Additionally, the sperm DNA methylome was profiled with whole-genome enzymatic methylation sequencing (EM-seq). EM-seq is a novel alternative to whole genome bisulfite sequencing that displays higher efficiency and sensitivity due to enzymatic-based conversion steps that minimize damage to the template DNA. The average proportion of total CpG methylation in the studied sperm samples was 85.6% (sd=0.6%). A weighted correlation network approach was employed to identify comethylated regions associated with the recorded traits. The analysis yielded a number of significant associations (at Bonferroni-corrected 5%). Functional annotation and enrichment analysis of gene sets assigned to such regions revealed terms that could be affiliated to various aspects of spermatogenesis such as cadherin- dependent signal transduction, cytoskeleton-related processes and endocrine function. Overall, the results suggest that sperm quality may be epigenetically regulated.

Keywords: DNA methylation; epigenomics; EM-seq; fertility; sperm motility

UNRAVELING SEX-DEPENDENT EPIGENETICS MECHANISMS IN RESPONSE TO ENVIRONMENTAL CUES

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SUMMARY

In the context of climate change, cultured fish face two environmental challenges: increased water temperature and the spread of infections. Over the past decade, emerging data have flourished in revealing the underlying epigenetic mechanisms of fish phenotypes. Here, experimental data from fish exposed to various environmental factors were analyzed using state-of-the-art methodologies (e.g., miRNA- and RNA-sequencing, Whole Genome Methylation Sequencing, WGMS, or Methylation Bisulfite Sequencing, MBS). To decipher informative molecular markers acting as epirecorders of climate change and infection insults, data on epigenetic mechanisms in European sea bass (*Dicentrarchus labrax*) and zebrafish (*Danio rerio*) were identified. For instance, transcriptomic data of E. sea bass gonads 48 hours after infections revealed a greater number of differentially expressed genes (DEGs) in testes than in ovaries and the expression of four miRNAs in the testis was detected as a recorder of past events. To deeply understand the crosstalk between reproduction and immune systems, *in vitro* experiments with dre-miR-210 in zebrafish gonadal cells were conducted. Results showed transcriptomic alterations in the gonadal cells, with the inhibition of reproduction-related pathways (e.g., oocyte meiosis) and upregulation of some immune-related pathways (e.g., immune response). In conclusion, our data suggest the importance of sexual dimorphism in epigenetic events, indicating that sex is an essential factor that needs consideration in cultured fish.

Keywords: Immune, Reproduction, Epigenetics, Infection, microRNA

Session 4:
Development and early life interventions

SALMOCODE: A SINGLE-CELL ATLAS OF ATLANTIC SALMON ORGANOGENESIS

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SUMMARY

As they develop in eggs exposed to the environment, fishes are especially susceptible to external influences during embryogenesis. These include temperature and light, which have been shown to have long-term fitness effects. In the SALMOCODE project, we will use single-cell transcriptomics to investigate these vulnerabilities in the Atlantic salmon embryo.

As a first step, we have studied the earliest phases of organogenesis in Atlantic salmon using single-cell transcriptomics. By isolating nuclei from four early developmental stages (90–150 day degrees, corresponding to the interval between gastrulation and mid-somitogenesis) and profiling them using a split-pool barcoding protocol, we have collected a dataset for 4864 embryonic cells.

During gastrulation only four cell types are represented (endo-, meso-, and ectoderm, and enveloping layer), however these rapidly differentiate into at least 25 distinct cell types in the last sampled stage. The high temporal resolution enables the identification of clear differentiation trajectories for the emergence of future tissues and organs, including the neural crest, the heart, and pronephros. In addition, the dataset already allows the discovery of key regulatory genes involved in the earliest cell fate commitments for these tissues.

We are currently expanding this effort to become a reference atlas of the salmon embryo from gastrulation up until hatching. In addition, we are using CRISPR/Cas9 gene editing to verify the involvement of several genes we identified in heart development.

Keywords: development, embryo, Atlantic salmon, scRNA-seq, atlas

EPIGENOMICS OF EMBRYOGENESIS IN THE TURBOT (*Scophthalmus maximus*)

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SUMMARY

Uncovering the epigenomic basis of embryogenesis is essential for a deeper understanding of the mechanisms underlying growth, environmental adaptation, and gamete quality, as a key tool to improve selective breeding. Moreover, a comprehensive characterization of genomic regulatory elements is a much-needed tool to aid in the identification of genetic variants potentially associated with valuable phenotypes to improve genomic predictions in breeding programs. In this work we developed an epigenomic atlas of the turbot genome during embryonic development, by integrating genome-wide datasets for ATAC-Seq, histone ChIP-Seq (for H3K4me3, H3K27ac and H3K27me3) and RNA-Seq using 54 pools of embryos in six developmental stages: high blastula, embryonic shield, early-, mid- and late-segmentation and prehatching. We first inspected the consistency of the epigenetic signal by checking its correlation between assays across developmental stages using a hierarchical clustering heat map. Our goals were to: i) characterize gene expression and GO profiles for each stage and across development; ii) define a chromatin state model by combining transcriptomic and epigenomic information; iii) identify and characterize regulatory elements (promoters/enhancers) and their clustering across the genome (super-enhancers); iv) check for the correlation of epigenomic and transcriptomic data; and v) characterize transcription factor (TF) binding motifs enriched in active promoters / enhancers regarding the expression profiles of the TFs. This multi-omic investigation of the turbot embryogenesis advances our understanding of the epigenomic basis for teleost development and provides novel genomic regions to target in future precision breeding applications.
This study was funded by the AQUA-FAANG (H2020 Grant Agreement No 817923).

Keywords: Epigenetics, Chromatin, Transcription factor, Enhancer, Turbot

DNA METHYLATION DURING EARLY DEVELOPMENT IN DIPLOID AND TRIPLOID EUROPEAN SEABASS

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SUMMARY

The induction of triploidy is used in aquaculture of some fish and mollusk species to optimize growth and mitigate issues associated with sexual maturation. In fish, induced triploidy can be achieved by retaining the second polar body through physical shocks. This results in the sterility of the triploids, particularly females, and impacts the survival and development of embryo. However, our understanding on the underlying epigenetic mechanisms remains limited, and conflicting results exist in the literature. Therefore, our objective was to explore the effects of triploidization on DNA methylation, during embryo development of European sea bass (*Dicentrarchus labrax*). The 90% epiboly and hatching stages were analyzed. About 30,680 differentially methylated cytosines (DMCs) were identified as either hyper- or hypomethylated in both stages, when compared to the corresponding diploids. These DMCs were allocated along the entire genome, but mostly in intergenic regions and CpG islands. The distribution of methylation differences reveals that around 25% of the total DMCs exhibit the highest possible methylation difference (> 80%) compared to diploids. Possible transcriptomic consequences associated to the identified methylation differences in the DNA of triploids, will be investigated. Accordingly, RNA sequencing was performed, generating around 30 million reads for each sample. Bioinformatics and statistical analysis will be conducted on four replicates for each stage and ploidy level, to identify Differentially Expressed Genes (DEGs) and to examine whether they correlate with the observed epigenetic changes. These results will provide novel information on how gene expression is regulated with the triploidy-induced presence of an extra maternal genome.

Keywords: Triploidization, development, sterility, DNA methylation, transcriptome

INTEGRATION OF MULTI-OMICS DATA TO UNCOVER KEY GENES AND METABOLIC PATHWAYS INVOLVED IN FLATFISH METAMORPHOSIS

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SUMMARY

Regulating gene expression is crucial to the intricate processes of flatfish metamorphosis, which orchestrates dynamic changes during the transition from pelagic to benthic lifestyle. Flatfish metamorphosis involves significant modifications in both form and function. The coordination of epigenetic mechanisms, such as DNA methylation, histone modification, and chromatin remodelling plays a crucial role in regulating gene expression during this transformative process. This ultimately results in the emergence of the juvenile body plan. This study investigates flatfish metamorphosis by integrating omics data that provide information on chromatin status, DNA methylation profile and gene expression in three key stages of turbot (*Scophthalmus maximus*): pre-metamorphosis, climax and post-metamorphosis. DNA methylation profile analysis showed no significant changes in global methylation levels in the three key stages. However, we found a significant number of differentially methylated regions (DMRs), specifically at the metamorphic climax stage, when compared to the pre-metamorphic and post-metamorphic stages. Furthermore, our investigation revealed the presence of DMRs during climax coinciding with regions of accessible chromatin. Of note, using an outside-in analysis approach, we discovered that 85% of the identified DMRs were located in regions of accessible chromatin within CpG islands. Of these regions, 78% colocalised with promoters, identifying 22% as orphan CpG islands. Finally, by integrating gene expression data, we identified genes susceptible to methylation-mediated repression, thereby elucidating the regulatory impact of methylation on gene expression during flatfish metamorphosis.

Keywords: Flatfish, Metamorphosis, Epigenetic, Omics

IMPROVING ATLANTIC SALMON HEALTH AND WELFARE BY EMBRYONIC TEMPERATURE PROGRAMMING

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SUMMARY

It has become clear that the environment encountered during critical periods of embryogenesis can have long-term effects on the resulting phenotype. The embryonic environment that facilitates fast growth may result in undernutrition during early life, and potentially negatively alters life-long metabolic programs. In the COOLFISH project, we aim to define the role and mechanistic basis for programming by embryonic temperature on the development and function of the Atlantic salmon immune system later in life, and exploit this knowledge to enhance fish health, welfare and production. Using multi-level analyses (i.e. epigenomic, transcriptomic, single-cell transcriptomic, proteomic, metabolomic, and immunological) at early developmental, parr and post-smolt stages, in-depth knowledge will be provided on the physiological and integrated molecular mechanisms driving the interaction between the embryonic rearing environment, organ development, growth and immune system function. Salmon exposed to different embryonic temperatures displayed a differential response towards a bacterial challenge with *Yersinia ruckeri* prior to start feeding as well as in juvenile stages. In salmon fry liver, cell-type specific embryonic temperature- dependent differences in gene expression, including genes and pathways involved in growth, metabolism and immunity, were revealed using single cell transcriptomics. We are currently working on detailed analyses of the infection dynamics to discover the molecular drivers of metabolic programming of immune function by embryonic temperature.

Keywords: Atlantic salmon, Metabolic programming, Single-cell sequencing, Multi-omics, Immune function

CORRELATION BETWEEN EPIGENETIC AND TRANSCRIPTOMIC CHANGES IN GOLDEN BARRAMUNDI (*LATES CALCARIFER*): A MULTIOMIC APPROACH

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SUMMARY

In many aquaculture species, coloration is an important trait, especially when rare color phenotypes are economically valuable. The barramundi (*Lates calcarifer*) is a significant aquaculture species across its Indo-Pacific range. The wild-type (WT) skin color of barramundi is typically silver to bronze, however, occasional sightings of golden (xanthic) variants are observed and are of commercial interest.

In this study, a comprehensive analysis of whole-genome bisulfite sequencing (WGBS) DNA methylation and RNA sequencing (RNA-seq) transcriptome data was conducted to investigate the molecular mechanisms underlying pigmentation in golden barramundi. Through a multiomics approach, we have found several genes that had a correlation between expression ratio and methylation percentage, highlighting the role of DNA methylation in regulating gene expression in the golden variants. Notably, methylation and expression levels of genes involved in pigment production, such as *tyrp1* and *pax7a*, were found to be significantly correlated.

After this investigation, it was hypothesized the phenotypic coloration in barramundi is likely the result of multiple molecular changes, rather than being solely influenced by a single gene. Furthermore, in this investigation the whole genome methylation profile of barramundi was examined for the first time, which is one of the few to analyze changes in the transcriptome and methylome associated with skin pigmentation in teleosts.

By elucidating the role of methylation in the onset of rare coloration in barramundi, this study enhances our understanding of the broader role of methylation in vertebrate coloration changes and provides valuable insights into molecular and biological processes.

Keywords: Methylation, RNA-Seq, pigmentation, Asian seabass, multiomics

Session 5:
Structural genomics and population genetics

BROODSTOCK MANAGEMENT INFLUENCES THE METABOLIC STATUS, GENE EXPRESSION AND EPIGENETIC GENE REGULATION IN PROGENY

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SUMMARY

In the Atlantic salmon farming sector, adjusting the spawning season of female broodstock is a widespread strategy to ensure a steady supply of offspring all year-round. Environmental factors such as water temperature, light exposure, and feeding schedules are manipulated to optimize reproductive timing. Therefore, gaining a deeper understanding of the biological mechanisms influenced by these alterations is crucial for improving the growth performance of the offspring, as they are important as the production fish in aquaculture.

In this study, we examined in detail four different spawning seasons achieved through two different production methods from commercial production in Norway: recirculating aquaculture systems (RAS) and sea-pen-based broodstock. Alongside the normal spawning season in sea-pens in November, we analysed three adjusted seasons: an off-season (five-month advance in RAS), an early season (two-month advance in sea pens), and a late season (two-month delay in sea pens). Previous findings have demonstrated that the altered spawning seasons significantly affect both the nutritional status of broodstock and offspring, thereby impacting nutrient transfer and offspring development. Here, we show significant changes in metabolic profiling, the patterns of gene expression (RNA-seq method) and DNA methylation (RRBS method) due to the altered spawning seasons. Offspring from RAS-based off- season spawning exhibited reduced larval weights and displayed changes in 1C metabolism genes and lipid-mediated regulations. Both early and late seasons impacted cellular processes, particularly cell cycle regulation. Here we show metabolic and epigenetic consequences in offspring due to broodstock management practices. We suggest studying the critical stages of broodstock development during which they are responsive and sensitive to management practices, thus impacting the above findings in offspring phenotype.

Keywords: Broodstock, Spawning season, Intergenerational epigenetics, Metabolomics

FIRST INSIGHTS INTO GENETIC DIFFERENTIATION AMONG SYMPATRIC MORPHS OF ARCTIC CHARR BASED ON WHOLE-GENOME SEQUENCING

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SUMMARY

The aim of this study was to document genetic differentiation among sympatric morphs of Arctic charr (*Salvelinus alpinus*) using low-coverage (2.5 X), whole genome sequencing data. The experimental design involved phenotypically characterized large and dwarf benthic, and pelagic planktivorous (PL) and piscivorous (Pi) morphs from two Icelandic lakes (Mývatn and Thingvallavatn) and dwarf benthic and pelagic morphs from two lakes in Norway (Vangsvatnet and Sirdalsvatnet). The sample size of each population was *n* = 22 - 40. Principal component analysis revealed that populations clustered by lakes and not by morphs, consistent with founder effects when the lakes were colonized. The pairwise comparison between the dwarf benthic and pelagic morphs showed no significant genetic differentiation in Vangsvatnet. In sharp contrast, high genetic differentiation was observed across the entire genome between these morphs from the lake Sirdalsvatnet. Significant genetic differentiation at many loci was also revealed when comparing the two benthic with the two pelagic morphs from lake Thingvallavatn. We identified two putative inversions that differentiate locally adapted benthic and pelagic morphs from this lake. Morph- specific haplotype signatures detected in Thingvallavatn was not replicated when comparing corresponding morphs from the other lakes, demonstrating that genetic differentiation among morphs does not have a common origin but has occurred independently in different lakes. However, it should be noted that this preliminary analysis is based is using a reference genome from a closely related species (*Salvelinus malma*, GenBank: GCF_002910315.2) which means that some regions of the genome could be poorly covered. We are currently reanalysing the short-read data using a recently completed high-quality assembly of the *Salvelinus alpinus* genome.

Keywords: Arctic charr (*Salvelinus alpinus*), sympatric morphs, whole-genome sequencing, genetic differentiation

UNVEILING GENOMIC SIGNATURES FOR DOMESTICATION IN EUROPEAN SEABASS (*DICENTRARCHUS LABRAX* L.)

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SUMMARY

The genetic basis of performance traits in cultured fish is of paramount importance in aquaculture research. Genome scans allow for the comprehensive exploration of underlying genetic markers and variations associated with desirable traits, providing valuable insights into the molecular mechanisms governing the performance characteristics crucial for selective breeding and enhancing aquaculture productivity. In this study, we contrasted ca. 310K SNPs between 12 wild and 12 farmed European populations of European seabass (*Dicentrarchus labrax* L.) to uncover specific genetic signatures associated with the domestication process. Remarkably, novel highly differentiated regions were detected. These regions were further annotated and analyzed, revealing potential key genetic elements contributing to the adaptation of European seabass to aquaculture conditions. In this context, we discuss the functional role of these identified genetic regions as well as their implications for the selective breeding of European seabass in aquaculture. These findings highlight the intricate interplay between genetics and environmental pressures in shaping the genomic landscape of cultured fish, offering promising avenues for targeted genetic improvement strategies in aquaculture.

This study was conducted under the project "SystEms Biology Modelling of Key LIFe History Traits for Sustainable Aquaculture Production in the Mediterranean Region'', funded by H.F.R.I.

Keywords: artificial selection, genome scan, single nucleotide polymorphisms (SNPs), sustainable aquaculture

MITOCHONDRIAL FUNCTION IS AT THE FRONTLINE OF DOMESTICATION IN EURASIAN PERCH (*Perca fluviatilis*)

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SUMMARY

Domestication exerts a profound influence on finfish aquaculture by selecting for traits conducive to survival and productivity within controlled environments. This selective process engenders both molecular and phenotypic modifications that manifest across reproductive and early developmental phases, evidenced by variations in egg composition, larval performance, digestive efficiency, and immune competence. Leveraging transcriptomic profiling of larvae, our research offers an understanding of the phenotypic diversity observed within and across populations in the context of domestication. In this investigation, we undertook a comprehensive transcriptomic analysis of freshly hatched Eurasian perch larvae, drawing from six distinct populations (comprising three domesticated and three wild populations; 5 families per population), species constituting excellent model for studying domestication in freshwater finfish of commercial significance. Our analysis of 123 differentially expressed genes (DEGs) between domesticated and wild populations unveils a multifaceted genetic response to domestication, influencing key processes such as growth, development, stress tolerance, and disease resistance. Further scrutiny of 1,113 genes, differentially expressed in pairwise comparisons between domesticated and wild populations (hereinafter considered as domestication- sensitive genes; DSGs), revealed 223 genes associated with mitochondrial functions. Further enrichment analysis of DSGs identified mitochondrial translation as a critical process susceptible to domestication pressures. These findings illuminate the central role of mitochondrial functionality and energy metabolism in the domestication of Eurasian perch, positing significant consequences for aquaculture practices and selective breeding programs. This study, therefore, delineates the intricate transcriptomic alterations induced by domestication, providing valuable insights for the refinement of traits desirable in aquaculture via strategic genetic and management interventions.

Keywords: domestication, transcriptomics, larvae

DAY 3 | Friday 24 May

Session 6:

Nutrition, growth and pigmentation

TRANSCRIPTOMIC ADAPTATION TO SALINITY IN MOZAMBIQUE TILAPIA TAKES SHORTER TIME THAN IN NILE TILAPIA

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SUMMARY

This study investigates the impact of elevated salinity through a long-term experiment comparing the responses of Mozambique and Nile tilapia. The experimental protocol involved gradually exposing the fish to saline water over an 8-week period, incrementally reaching a concentration of 30 ppm. Repeated sampling of gills from the same individual fish was conducted throughout the experiment. RNA expression in the gills was analyzed for differential gene expression (DGE) and to identify Single Nucleotide Polymorphisms (SNPs).

Results from the Mozambique tilapia indicated 19,043 differentially expressed transcripts. Group- wise analysis revealed that the most significant differential expression occurred between 10 ppm and 20 ppm. Additionally, 28,493 SNPs were identified, with 191 SNPs showing Allele-Specific Expression (ASE). Of these, 20 ASE SNPs corresponded to differentially expressed transcripts between freshwater and 10 ppm, 25 SNPs between freshwater and 20 ppm, and 17 SNPs between freshwater and 30 ppm.

Our findings suggest that Mozambique tilapia exhibits a more rapid adaptation to increased salinity compared to Nile tilapia, which demonstrates a prolonged and delayed adaptive response.

Keywords: Nile tilapia, Mozambique tilapia, salinity challenge, gene expression, allele expression

CANDIDATE LIST OF BIOLOGICAL AGE MARKERS FOR WELFARE AUDITING IN FARMED GILTHEAD SEA BREAM

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SUMMARY

Attempts based on transcriptomics and epigenetic approaches arise as integrative biomarkers linking the quantification of stress response with a given fitness trait. Thus, although epigenetic or "DNA methylation" clocks were initially developed to track chronological age, accumulating evidence indicates that they bring the possibility of a high resolution and precise understanding of age-related pathology and physiology as deviations of biological age. Targeted analyses of gene expression and local DNA-methylation patterns in skeletal muscle demonstrated that *sirt1* is a reliable marker of age- and seasonally-mediated changes of energy metabolism in gilthead sea bream. The present study aimed to complete the list of welfare auditing biomarkers by the integration of wide-analyses of gene expression (RNA-seq) and DNA methylation (MBD-seq) in one- and three-year old fish. After partial least squares-discriminant analysis, 668 discriminant transcripts were matched with those containing discriminant differentially methylated (DM) regions (14,366), and 172 were overlapping. Up to 108 were retained after enrichment analyses. Among them, 33 transcripts showed an opposite trend for DNA-methylation and expression, while 61 transcripts (57 unique gene descriptions) were downregulated likely by a hypomethylation-induced repressive chromatin (gene instability). Genes filtered by a pluripotent function in both set of genes (14 + 26) were deemed potential biological age biomarkers. With focus on the first set of transcripts, 10 genes with differentially methylated CpG in regulatory gene regions (*sirt1*, *smad1, ramp1, psmd2* – up-regulated; *col5a1, calcrl, bmp1, thrb, spred2, atp1a2* – down-regulated) were proposed as robust biological age markers for an improved welfare auditing in farmed gilthead sea bream.

Keywords: Biological age, DNA methylation, epigenetics, transcriptomics, welfare.

A LA CART(E) GENOME-EDITING FOR ENHANCED FEEDING BEHAVIOR IN NILE TILAPIA

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SUMMARY

The use of fishmeal in cultured-fish feeds accounts for increased criticisms due to its low sustainability and high cost. Although plant-based diets can be formulated, their palatability to fish are low, leading to the continuous use of fishmeal also for feeding herbivorous and omnivorous fishes. The omnivorous Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured species in global aquaculture. Hence, enhancing tilapia feeding behavior may help to improve aquaculture by removing fishmeal from their diet. Cocaine- and amphetamine-regulated transcript (CART) is a pleiotropic neuropeptide involved in the central regulation of stress, anxiety, depression, reproduction, and circadian functions. However, it is mainly known for its regulation of body weight and appetite. While mammals possess a single *CART* gene, fish may possess multiple paralogous *cart* genes. In the present study, we identified seven *cart* genes in the brain of Nile tilapia. Acute starvation of adult tilapia significantly decreased the hypothalamic expression of four *cart* genes, and two of them also displayed suppressed hypothalamic expression in response to chronic starvation. We then utilized the CRISPR/Cas9 method to generate germline tilapia carrying loss of function mutations in the primary appetite-regulating *cart* gene and analyze their outcome in feeding-related behavioral assays.

Keywords: Nile tilapia, Cocaine- and amphetamine-regulated transcript, genome-editing, behavioral analysis.

EARLY LIFE FEEDING EFFECTS ON NILE TILAPIA GUT MICROBIOME AND TRANSCRIPTOME

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SUMMARY

Initial microbial colonization in animals appears to shape the gut microbial community with lifelong effects on, for example, health and metabolism. We are only beginning to understand how variation in the history of the initial colonization through feed or the environment may shape gut development and host performance. Especially in fish, information is still lacking. We applied 16S rRNA and transcriptome sequencing using Oxford Nanopore technology to explore the gut microbiome and host response of Nile tilapia larvae, exposed to different diets containing different levels and types of beta-glucans (soluble vs insoluble). We assessed the efficacy of using long-read sequencing technology with the Nanopore platform to study changes in the gut microbiome, compared to the current standard approach with Illumina sequencing. Moreover, to understand the effects of beta-glucans on gut development and immune response, we evaluated the whole gut transcriptome during the first three weeks of feeding. Our results show that development shapes largely the microbiome composition during the early life stages, while beta-glucan effects relate to their type and inclusion level in the diet. An increased expression of immune-related genes was observed after one week of glucan administration; however, this was reversed during continuous feeding with glucans for three weeks. A better understanding of early life feeding and how this impacts fish immune response and microbiome development is a great challenge for the improvement of hatchery protocols to ensure better fish survival and performance in the long-term.

Keywords: Diet, Gene expression, Gut Health, Larvae, Immune response

GWAS AND ACCURACY OF PREDITION FOR GROWTH IN MEAGRE *ARGYROSOMUS REGIUS*

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SUMMARY

Meagre (*Argyrosomus regius*) is a fast-growing sciaenid species which recently received a rising interest in Mediterranean aquaculture diversification and industry sustainability. Genomic selection has become popular in other Mediterranean species like the European sea bass and the gilthead sea bream but for meagre these modern animal breeding approaches have not yet been adopted. In the present study, 633 fish at the age 770 and 978 days-post-hatching were weighed and the growth was calculated as the difference between the measurements. Fin-clips were collected and genotyped using the ddRAD-seq methodology and after quality control, 4,573 SNPs passed and participated in the following analyses. A GWAS was performed to identify QTL linked with the total growth revealing one QTL on chromosome 17, explaining approximately 3.25% of the total phenotypic variance. Furthermore, a comparison between the accuracy of prediction of the growth (by masking the 20% of the phenotypes of the total population, repeated 20 times) using the pedigree relationship matrix (PRM) and the genomic relationship matrix (GRM) was performed. The accuracy of prediction was estimated as the correlation between the predicted breeding value and the phenotype. The average accuracy of prediction was 0.56±0.05 and 0.62±0.05 using the PRM and the GRM, respectively. The *p*-value of the *t*-test between them was 5.14×10^{-7}, showing a statistically significant difference. Present findings provide favorable results showing the superiority in the use of genomic information, especially when low-density SNP coverage is used for the improvement of a main breeding goal like the growth in meagre.

Keywords: *Argyrosomus regius,* accuracy of prediction, growth, GWAS

Acknowledgements

The study received funding from the Hellenic Republic through the "MeagreGen" project under the call "Special Actions – AQUACULTURE" in the Operational Program "Competitiveness Entrepreneurship and Innovation 2014-2020".

IDENTIFICATION OF SNPs AND CANDIDATE GENES ASSOCIATED WITH GROWTH USING GWAS AND TRANSCRIPTOME ANALYSIS IN *Coilia nasus*

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SUMMARY

The improvement of growth trait can foster the sustained development of the aquaculture industry and is generally regarded as a primary objective in the breeding projects for economic fish. *Coilia nasus*, a migratory fish species naturally inhabiting in the middle and lower reaches of the Yangtze River and offshore waters of China, exhibits significant culturing potential and economic value. However, due to genetic degradation, there is a pronounced trend towards miniaturization and exaggerated growth variations within the cultured population. In order to facilitate the healthy development of the aquaculture industry of *C. nasus*, whole-genome association analysis (GWAS) and transcriptome analysis (RNA-seq) have been employed to identify molecular markers associated with growth traits and elucidate genetic foundations. A total of 234 individuals of *C. nasus* were subjected to whole-genome resequencing for genotyping, resulting in the identification of 2,306,254 high-quality SNPs. Fourteen SNPs were proved to have suggestive association with body length (BL) or body weight (BW). We obtained two shared significant SNP peaks for BL and BW on LG6 and LG9, and *bmp8a* and *uqcrfs1* were identified as their causal genes. Comparative transcriptome analysis based on extreme body size obtained 54 and 20 overlapping differentially expressed genes (DEGs) between different sexes in brain and muscle, respectively. Combining the results of GWAS and RNA-seq, we identified five candidate genes and one pathway associated with growth, including *uqcrfs1*, *bmp8a*, *gabrb4*, *tshz3a*, *ahcyl1*, and the mTORC1 signaling pathway. Our research findings provide molecular markers for genetic selection in *C. nasus* growth.

Keywords: *Coilia nasus*, GWAS, transcriptome, growth, SNP

UTILIZING POOL-SEQ TO IDENTIFY THE GENE CONTROLING BLACK PATCHES PIGMENTATION IN ORNAMENTAL CARP (KOI)

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SUMMARY

The Japanese ornamental carp (*Cyprinus carpio*, var. Koi), has an extraordinary repertoire of color combinations and patterns, derived from the uniformly colored common carp. Thus, Koi makes an excellent model for studying pigmentation genetics. Despite commercial and scientific interest, the genetics underlying this coloration remain poorly understood. The 'bekko' pattern is characterized by black patches over a white/red body. Phenotypic segregation of this pattern in families suggested a single-gene dominant mode of inheritance, with possible selection against homozygous dominant individuals. After establishing the mode of inheritance, we continued to study the genetic basis of this phenotype. First, we followed the development of 'bekko' phenotype during fry development in multiple families involving 'bekko' parents. Upon hatching, all offspring lack black pigmentation and the proportion of black-pigmented individuals gradually rises with age until reaching ratios of 1:1 or 3:1 in 'bekko' x 'non-bekko' or 'bekko' x 'bekko' families, respectively. In 'bekko' x 'bekko' families, the ratio keeps changing and reduces to 2:1, in concordance with the differential survival finding. Next, we applied genome re-sequencing of DNA pools from 'bekko' and 'non-bekko' fish from various sources and genetic backgrounds. A single, narrow, and highly significant QTL was identified, containing a promising pigmentation candidate gene. To validate this identification, markers within this QTL were genotyped in individuals across families and populations. Our results demonstrate the efficiency of pool-seq for mapping single-gene traits. Unraveling the genetics of pigmentation will promote selection of Koi broodfish with high commercial value and enhance our knowledge of pigmentation biology.

Keywords: Black pigmentation, pool-seq, Cyprinus carpio Koi fish, pigmentation genetics

GENOME SURVEY FOR PROCESSES SUSCEPTIBLE TO MANIPULATION THROUGH DNA METHYLATION REMODELING IN GILTHEAD SEA BREAM

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SUMMARY

More knowledge is required to exploit fish plasticity to obtain phenotypes that better match aquaculture and market conditions. To search for processes susceptible to manipulation through epigenetic interventions, pituitary and liver explants of *Sparus aurata* juveniles were exposed to the DNA methylation inhibitor 5-aza-2'-deoxycytidine (DAC) for 24 h. Then, changes in genome-wide gene expression (RNA-seq) and DNA methylation (RRBS) were assessed. As expected, major differences in gene expression were observed between tissues. No differentially expressed genes (DEGs) were observed in pituitaries exposed to DAC, but Rank-Based Gene Ontology Analysis with Adaptive Clustering (GO-MWU) revealed 87 GO Biological Processes and 23 GO Molecular Functions potentially affected. In liver, DAC produced 60 DEGs (adjusted p-value <0.05, FC cutoff of log2fold = 0.585), though 120 genes exhibited significant changes when the log2fold cutoff restriction was removed. Twenty-five GO Biological Processes and 4 GO Molecular Functions were potentially affected in liver. Liver and pituitary methylation landscapes were remarkably different, and DAC produced more differentially methylated cytosines (DMCs) and regions (DMRs) in pituitary (5087 DMC; 370 DMRs) than in liver (318 DMC; 26 DMRs). DMCs were significantly enriched at exons, CDS, introns, 3'UTRs and promoters in both tissues. Interestingly, DAC effects on DNA methylation of each tissue differed, with demethylation occurring in most differentially methylated genes (DMGs) in liver as expected, while in pituitary most DMGs underwent hypermethylation. Relationships could be described for DNA methylation and gene expression, with half of the DEGs in liver being also differentially methylated. PID2021-128540OA-I00 funded by MCIN/AEI/10.13039/501100011033 + ERDF.

Keywords: DNA methylation, *ex vivo* culture, epigenetics, fish, tissue explant

POSTER PRESENTATIONS

P3- DECIPHERING THE IGH LOCUS OF GILTHEAD SEABREAM

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SUMMARY

Antibodies are composed of two heavy (H) chains and two light (L) chains encoded by the IGH locus and IGL locus, respectively. Teleost IGH locus has, as in mammals, a translocon configuration, characterized by multiple IGHV, D, and J gene segments, followed by a series of IGHC genes encoding the IgT, IgM and IgD antibodies. The diversity of antibodies required to recognize every possible antigen is mainly achieved through the somatic recombination V/D/J segments that happens during the B cell development. Thus, differences in teleost IGH loci have a major effect on determining the diversity of antibodies available in a host species and can involve unknown consequences for the immune response. The aim of the present work was to undertake a full annotation of the IGH locus in gilthead seabream (*Sparus aurata*), a relevant Mediterranean aquaculture species. To this end, we used the high-quality gilthead seabream genome (fSpaAur1.1) assembly, available IGH sequences and IMGT rules and standards. The gilthead seabream IGH locus is located on chromosome 23. It comprises two sets of IgT, IgM and IgD encoding genes in tandem duplication, separated by VH-containing regions. Interestingly, at least five copies of IgT encoding genes were identified, and the Cδ2-Cδ3-Cδ4 domains are repeated eight times in the IgD encoding genes. More than 260 VH, 50DH and 20JH genes were identified and annotated. Besides the great value in comparative immunology, the annotation of the gilthead seabream IGH locus will provide a reference data set for studying immunoglobulin responses in gilthead seabream.

Keywords: Immunoglobulins, *Sparus aurata*, IGH locus, annotation.

P4- NUTRITION AND IMMUNE FUNCTION IN FARMED FISH REGULATION OF INFLAMMATORY AND ANTI-INFLAMMATORY RESPONSES

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SUMMARY

Infectious diseases cause economic losses in the aquaculture sector. Nutritional intervention has the potential to control inflammation and improve resistance to pathogens. Farmed salmon were traditionally fed a diet with high levels of fish oil and fish meal but supply of these nutritional components is unable to meet the global demand. Alternatives to fish oils are vegetable oils which are increasingly used in salmon feeds, but the impact of these diets on inflammation is not fully understood. Vegetable oils do not contain omega-3 LC-PUFA but are rich in omega-6 PUFA, hence fish fed vegetable oils have limited DHA and EPA, which are precursors for many immune regulatory molecules. The project will enhance our understanding of the effects of dietary omega-3 on inflammation in farmed Atlantic salmon (*Salmo salar*), with a focus on the mRNAs encoding enzymes involved in pro- and anti-inflammatory eicosanoid lipid mediators. Our initial steps have been to characterise several of the key genes involved in these pathways and examine the expression of different paralogues following immune response. For this RNA was extracted from head kidney tissue of Atlantic salmon stimulated with either heat killed *Vibrio* or poly I:C to induce an antibacterial or antiviral response respectively. Eicosanoid pathway involvement in inflammatory responses was indicted by elevated COX2a2 transcription stimulated by vibrio alongside a decline in ALOX5a1 transcription. Future work in this project will analyse the transcriptomic effects of alternative dietary oils in vivo and in cell cultures using paralogue specific primers.

Keywords: Inflammation, Eicosanoid, omega-3, cox2a2, alox5a1

P5- DIFFERENT SAMPLING APPROACHES FOR DNA METABARCODING ANALYSIS OF THE STOMACH CONTENTS OF THE ATLANTIC BLUEFIN TUNA (THUNNUS THYNNUS LINNAEUS, 1758)

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SUMMARY

Atlantic bluefin tuna (BFT), *Thunnus thynnus* Linnaeus, 1758, is an important marine predator and an important target species for fisheries and aquaculture, especially in Croatia, where 85 % of commercial catches are destined for aquaculture. Studies on feeding ecology are important for a better understanding of their trophodynamics and food webs. DNA extracted from gastric juice (GJ) and homogenate of stomach contents (HSC) of 41 BFT, including both juveniles and adults collected in the eastern Adriatic Sea, was subjected to amplification of a partial fragment of the COI gene region using universal primers for metazoans and sequenced using the Illumina platform. Bioinformatics results showed large differences in sequencing reads between the different sampling approaches, with ~60% of reads originating from the host in the case of GJ compared to ~34% in the case of HSC. The most abundant prey species in both sampling approaches were small pelagic fish (HSC ~44 %, GJ ~25 %), cephalopods (HSC ~12 %, GJ ~11 %) and demersal fish species (HSC ~7 %, GJ ~3 %). The HSC had greater taxonomic richness and resolution, resulting in 32 taxa and 34 OTUs being assigned at the species level, compared to 26 taxa and 29 OTUs in the case of the GJ. Also, nine unique taxa were detected in the HSC compared to three in the GJ. In conclusion, the data derived from the homogenized stomach contents were more accurate and thus more useful for predator-prey analysis than the data obtained from the gastric juice samples.

Keywords: DNA metabarcoding, COI, gastric juice, homogenate of stomach contents, Atlantic Bluefin tuna

P6- INFLUENCE OF 3'UTR VARIANTS ON GERM CELL-SPECIFIC GENE EXPRESSION IN ATLANTIC SALMON

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SUMMARY

Potential applications using mRNA are increasing and could be used for vaccines, protein and antibody delivery in aquaculture. Depending on the application the location and duration of the gene expression (within the organism) can be of great importance. mRNA stability, translational efficiency and location are to a large extent determined by their 5`and 3`untranslated regions (UTRs). The 3'UTRs of genes specifically expressed in primordial germ cells (PGCs) have critical roles for the stabilization and location of these RNAs. However, the 3'UTRs are not well conserved in the different PGC-specific genes. We will investigate how different nucleotide sequences originating from salmon germ plasm mRNAs can confer and improve localization, stability and translation of *in vitro* transcribed mRNAs in the germ cells.

Keywords: Atlantic salmon, mRNA, primordial germ cells, 3'UTR

P7- MUSSEL ANTIVIRAL TRANSCRIPTOME RESPONSE AND ELIMINATION OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV)

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SUMMARY

As filter-feeding bivalves, mussels have been traditionally studied as possible vectors of different bacterial or viral pathogens. The absence of a known viral pathogen in these bivalves makes it particularly interesting to study the interaction of the mussel innate immune system with a virus of interest. In the present work, mussels were challenged with viral haemorrhagic septicaemia virus (VHSV), which is a pathogen in several fish species. The viral load was eliminated after 24 h and mussels evidenced antiviral activity towards VHSV, demonstrating that the virus was recognized and eliminated by the immune system of the host and confirming that mussels are not VHSV vectors in the marine environment. The transcriptome activating the antiviral response was studied, revealing the involvement of cytoplasmic viral sensors with the subsequent activation of the JAK-STAT pathway and several downstream antiviral effectors. The inflammatory response was inhibited with the profound downregulation of MyD88, shifting the immune balance towards antiviral functions. High modulation of retrotransposon activity was observed, revealing a mechanism that facilitates the antiviral response and that had not been previously observed in these species. The expression of several inhibitors of apoptosis and apoptosis- promoting genes was modulated, although clear inhibition of apoptosis in bivalves after severe viral infection and subsequent disease was not observed in this study. Finally, the modulated expression of several long noncoding RNAs that were correlated with genes involved in the immune response was detected.

Keywords: Mussel, bivalves, transcriptome, innate immunity, viral infections

P8- TRANSCRIPTOMIC ANALYSIS OF TURBOT (*SCOPHTHALMUS MAXIMUS*) TREATED WITH ZYMOSAN A REVEALS THAT LNCRNAS AND INFLAMMATION-RELATED GENES MEDIATE THE PROTECTION CONFERRED BY B-GLUCANS AGAINST *AEROMONAS SALMONICIDA*

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SUMMARY

Aeromonas salmonicida is one of the most harmful pathogens in finfish aquaculture worldwide. Immunostimulants such as β-glucans are used to enhance the immunity of cultured fish. However, their effects on fish physiology are not completely understood. In the present work, we evaluated the effect of a single intraperitoneal (ip) injection of zymosan A on fish survival against *A. salmonicida* infection. A single administration of this compound protected fish against *A. salmonicida* challenge and reduce the bacterial load in the head kidney one week after its administration. Transcriptome analyses of head kidney samples revealed molecular mechanisms involved in the protection conferred by glucans and their regulation by long noncoding RNAs. The transcriptome profile of turbot exposed only to zymosan A was practically unaltered one week after ip injection. However, the administration of this immunostimulant induced significant transcriptomic changes once the fish were in contact with the bacteria and increased the survival of the infected turbot. Our results suggest that the restraint of the infection-induced inflammatory response, the management of apoptotic cell death, cell plasticity and cellular processes involving cytoskeleton dynamics support the protective effects of zymosan A. All this information provides insights on the cellular and molecular mechanisms involved in the protective effects of this widely used immunostimulant.

Keywords: β-glucans, *Aeromonas salmonicida*, immune response, transcriptomics, lncRNAs.

P9- GENETIC STRUCTURE OF NATIVE AND TRANSLOCATED FRESHWATER CRAYFISH *PONTASTACUS LEPTODACTYLUS* POPULATIONS FROM GREECE AND TURKEY

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SUMMARY

In Greece, a country which occupies significant position towards Mediterranean mariculture industry, the farming of crustaceans still remains underdeveloped. Apart from their important role as ecosystem scavengers, the freshwater crayfish species *Pontastacus leptodactylus* is characterized by high economic value and high export orientation from fisheries. The species' native range in Greece is located in Evros region. Nevertheless, many translocation have been conducted during the last decades, expanding its distribution range in several artificial and natural lakes of Northern Greece. In the present study the genetic composition of the Greek *P. leptoodactylus* populations was investigated for the first time using microsatellite markers. In total 132 crayfish individuals were collected from five populations, including three translocated populations from Northern Greek lakes, namely Vegoritida, Volvi and Polifitou, one native Greek population from Evros river, and one population from Eğirdir lake, Turkey. Based on the results, although there is a level of weak genetic differentiation among the translocated populations, they are structured together. The Fst values and the AMOVA revealed that translocated populations are genetically closer to Turkish population probably indicating their origin. The native Greek population originating from Evros river revealed the highest genetic diversity, emphasizing its high conservation priority. In conclusion, our results demonstrate that translocated Northern Greek *P. leptodactylus* populations are of Turkish origin, probably attributed on multiple anthropogenic restocking events.

Keywords: *Pontastacus leptodactylus*, Greece, microsatellites

P10- DISSECTING THE GENETIC BASIS UNDERLYING RESPONSE AGAINST SALMONID ALPHAVIRUS IN ATLANTIC SALMON

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SUMMARY

Pancreas disease (PD) poses a significant challenge to Atlantic salmon aquaculture, with disease outbreaks resulting in large production losses. Previous research has identified several genomic regions associated with PD resistance and substantial underlying additive genetic component. However, identification of the causal underlying variation remains elusive, likely due to the overlap between PD-associated loci and highly duplicated regions within the salmonid genome. We hypothesize that this duplication has led to inconsistencies in the earlier Atlantic salmon reference genome (ICSASG_v2), ultimately masking the causal variation. This study applied recent advances in Atlantic salmon genomics to explore the genetic landscape underlying PD resistance and identify genomic variation with possible functional implications. Association testing and haplotype analysis of 5,628 fish challenged either by intraperitoneal injection of salmonid alphavirus (SAV3) or infectious cohabitation confirmed narrow locations on chromosomes 3 and 7. Together, the two regions harbor several genes, including three in tandem duplicated gig1-like genes, abcc6.b and slc38a, which showed significant differences in expression profile between fish infected with high and low concentrations of SAV3. Finally, use of long-read sequencing to detect structural variation revealed complex variants overlapping the disease-associated regions, with particular interest in microsatellites (STRs) overlapping the transcription start site and exon of the three gig1-like genes. In conclusion, our study provides a comprehensive exploration of the genetic landscape for PD resistance. Our findings refine previous knowledge, while the improvement in genomic resolution, together with detection of complex genomic variation, introduces new considerations regarding the causal variation underlying pancreas disease in Atlantic salmon.

Keywords: Atlantic salmon, pancreas disease, salmonid alphavirus, structural variation, whole genome duplication

P11- MOLECULAR AND PHYSIOLOGICAL RESPONSES TO CHRONIC STRESS IN EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*

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SUMMARY

Fish in aquaculture are exposed to various husbandry stressors, yet the molecular responses to chronic stress have not been thoroughly examined. The aim of the present study was to examine the molecular responses in European sea bass' tissues after exposure to a validated chronic stress protocol, before and after the application of an additional acute stressor. To this end, sea bass of an average weight of 184.4 ± 38.0 g were divided into 4 x 250 L tanks. Two tanks served as controls in which the fish were left undisturbed during the experiment, while in the other two the fish were exposed to chronic stress by exposing them once per day to either (i) chasing with a net for 5 min, or (ii) confining them in 1/5 of the tank volume for 30 min. The protocol lasted for 11 days; on the 12th day all fish were sampled before (T0 fish) or 1 hour after exposure to an additional 5 min chasing and confinement (T1 fish). Blood, brain, liver, gills, and head kidney tissues were collected from control and stressed fish. Preliminary data revealed that chronic stress caused a reduction in the expression of *pomc* in the brain and of *gr2* in the head kidney of fish. Moreover, in chronically stressed fish exposure to an additional stressor caused a downregulation of the *gr1* and *gr2* expression in the liver. All the above provide new evidence on the molecular regulation of the HPI axis and its actions in chronically stressed fish.

Keywords: chronic stress; cortisol; European sea bass; glucocorticoid receptors; pomc

P12- CAN ATLANTIC BLUEFIN TUNA BE EFFICIENTLY SWITCHED FROM SMALL PELAGIC FISH TO PELLET FEED UNDER FARMING CONDITIONS?

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SUMMARY

The Atlantic bluefin tuna (BFT), *Thunnus thynnus* Linnaeus, 1758, is an important target species for commercial and recreational fisheries. In Croatia, 85% of commercial catches of BFT are destined for aquaculture, making it the most important aquaculture species in terms of production value in recent years. The aim of this study was to compare the transcriptomes and microbiomes of BFT fed with novel feed designed specifically for tuna and BFT fed with European pilchards, *Sardina pilchardus* (Walbaum, 1792). In addition, tissue samples were taken from wild-caught adult BFT for comparison. The initial body weight of BFT in the feeding trial averaged 10 kg and the trial lasted almost 2 years. Based on quality, 53 total RNA samples were selected for cDNA library preparation, which initially included 5 biological replicates per feeding treatment from two selected intestinal parts, pyloric caeca, and distal intestine, spleen and liver. Paired-end RNA-Seq was performed using the NovaSeq X Plus system. All selected tissues were paired as subsamples, including the microbiome, to allow for joint analysis. V4 16S sequencing was performed on 53 samples of intestinal contents, including technical replicates, using the NovaSeq 6000 system. Preliminary results indicate a negative influence on transcriptomes and microbiomes of pellet fed BFT, confirmed by a lower overall fitness and a 35.4 % decrease in weight gain. Nevertheless, the search for alternative and more sustainable feeds for BFT aquaculture that reduce pressure on small pelagic fish is of great importance, and these results are a step in this direction.

Keywords: nutrigenomic, sustainable feed, transcriptome, microbiome, *Thunnus thynnus*

P13- DIVERGENT EVOLUTIONARY PATHS AND ONTOGENIC REGULATION OF PROTEASES IN THE GENOME OF EUROPEAN SEA BASS (*Dicentrarchus labrax)*

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SUMMARY

Proteolysis, a fundamental process in cellular activities, intercellular signaling, and tissue remodeling, is intricately regulated by various genes encoding distinct proteases like calpains and cathepsins families. In the genome of European sea bass (*Dicentrarchus labrax*), both families are characterized by substantial paralog retention, nine calpain and four cathepsin genes, respectively, stemmed from the teleost-specific whole genome duplication (TSWGD), in cases followed by tandem duplications, whereas ten genes draw their origins from older whole genome duplications (WGDs) within the vertebrate lineage, showcasing different evolutionary fates. We hypothesized that the different evolutionary trajectories have shaped differentiated regulation mechanisms, and we checked this hypothesis through the comparative analysis of RNA sequencing data from different developmental stages. Fish larvae undergo a complex process of development, including extensive tissue remodeling, as the organ systems mature, and they undergo metamorphosis to attain their adult morphology. Indeed, different ontogenetic patterns were observed across the genes (i.e., *CTSDa, CTSLa, capn3a, capn14b*), and several paralogs were stage-specific (i.e., *CTSLb* and *capn3b*). Among gene family members, the expression of classical calpains and cysteine cathepsins exhibited the highest differentiation between developmental stages, pointing to a link between the mode of catalysis and the evolution of regulation, and adding further complexity to the mechanisms of sub-functionalization in calpain and cathepsin genes.

Keywords: Proteolysis, Ontogenic regulation; evolutionary fates, paralog retention, European sea bass

P14- SEROTYPE VARIATION AND GENOMIC CHARACTERIZATION OF *Streptococcus agalactiae* STRAINS ISOLATED FROM NILE TILAPIA

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SUMMARY

Streptococcus agalactiae is a Gram-positive bacterium responsible for severe infections in various animals, including fish. Its impact on Nile tilapia farming has been alarming, causing significant economic losses due to high mortality rates. This study focused on four *S. agalactiae* strains: E8-UEL, E9-UEL, Maranhão, and Recife, isolated from Nile tilapia exhibiting clinical signs of streptococcosis in different regions of Brazil. These strains were serotyped, and their DNA was sequenced and analyzed for genome annotation, virulence factors and cps operon diversity, multilocus sequence typing, phylogenetic relationships, and non-coding RNA prediction. The analysis revealed a high degree of genome plasticity across serotype Ib, E8-UEL, and E9-UEL strains and serotype III, Maranhão, and Recife strains. Our findings include the discovery of exclusive ncRNAs and mobile genetic elements, highlighting the significance of regulatory elements and genetic exchange in the evolution and adaptation of these strains. The results also raise concerns about the public health implications, particularly regarding the spread of the ST283 lineage and its potential zoonotic capabilities. The detection of vancomycin tolerance-related genes in the Maranhão genome indicates a possible shift in the antibiotic sensitivity profile of this pathogen. Overall, this study contributes to understanding the genetic diversity and pathogenicity of *S. agalactiae* in aquaculture. It supports the importance of continuous monitoring and research into the genomic evolution of this species to better understand its pathogenesis and to develop effective management strategies for infections in aquaculture and public health contexts.

Keywords: Comparative genomics, genome plasticity, genetic diversity, aquatic pathogen

P15- SHOULD WE REAR TILAPIA LARVAE IN THE DARK?

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SUMMARY

Nile tilapia is one of the top aquaculture species globally due to its rapid growth, salinity tolerance, ease of reproduction, and readiness to accept artificial feed shortly after yolk sac absorption. In fish farming, larval rearing is an essential part of the production to ensure a successful commercial operation. Nile tilapia females rear larvae in their buccal cavity until around 12 days when the yolk sac is fully absorbed and the larvae are free swimming. In hatcheries, eggs are typically collected every five days and sorted by developmental stage. Because of the nature of maternal care in tilapia, we hypothesized that larvae will respond differently to light cues during larval development to cope with changes in environmental illumination (i.e., from incubation in the mouth to free swimming). Here, we assessed responses to light-dark conditions of tilapia larvae in different developmental stages and identified a developmental inversion in their response to light cues. Aiming to gain better mechanistic understanding of this dramatic change in behavior, we used single-cell RNA-seq of the entire brain and bulk RNA-seq of the retina of larvae collected before and after this behavior switch. Our findings demonstrate developmental cell differentiation in larval brains and developmentally altered retinal gene expression. Our results challenge the current convention on tilapia larval rearing and emphasize the importance of illumination conditions in hatchery operations.

Keywords: Nile tilapia, larvae, behavior, development, single-cell RNA-seq, transcriptomics

P16- COMBINED QUANTITATIVE AND POPULATION GENETICS METHODS PROVIDE INSIGHT INTO THE GENETIC LANDSCAPE OF FILLET FAT IN EUROPEAN SEA BASS *DICENTRARCHUS LABRAX*

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SUMMARY

The European seabass (*Dicentrarchus labrax*) plays a crucial part in the Mediterranean aquaculture industry, constituting the second most extensively farmed species. Consequently, challenges in commercial breeding for traits sharing strong negative genetic correlation such as growth and fillet fat deposition can have detrimental economic implications. This study investigated genetic paths linked to fillet fat deposition using a population of 520 European seabass, fed with two different diets, a plant-based and a standard commercial one. Fin-clips from all samples were collected and genotyped using the MedFISH SNP-array (Peñaloza et al., 2021) and chemical fillet fat for each sample was recorded at 665 Days Post Hatching. A univariate animal model for the fillet fat was used, fitting the diet (2 levels) as a fixed effect, to estimate genomic breeding values (GEBVs) in BLUPF90. Then, an F_{st} analysis of 60 fish with the highest and lowest GEBVs detected more than 931 SNP with significant genetic differentiation (F_{st}>0.2) between two groups (high and low GEBVs) using PLINK. Alignment of F_{st}-filtered SNP against functional information, and gene set enrichment analysis of SNP overlapping genes detected significant over-representation of biological processes involved in growth, development, energy and lipid regulation. Moreover, several genes with known involvement in fat metabolism such as *ffar3* and *acsl6* were found, for which variant effect prediction (VEP) analysis detected putative functional impact through changes in amino acid sequence.

Keywords: Fst, VEP, chemical fillet fat, European sea bass, gene enrichment

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P17- COMPARISON OF TENDON TISSUE DIFFERENTIATION BETWEEN ZEBRAFISH AND MEDAKA BY SINGLE-CELL SEQUENCING

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SUMMARY

Tendons belong to the dense connective tissue that connects muscle to bone and can transmit force between muscle and bone by using their biomechanical properties to store and release energy. In fishes, tendons are divided into two types: cranial tendons and axial tendons. Interestingly, in some special fish such as zebrafish, partial axial tenocytes naturally transform into osteoblasts and form bone tissue, known as intermuscular bone (IBs). But it's not present in the medaka. The development of IBs or tendon ossification is still not fully understood and the molecular basis of that remains to be established. Therefore, in this study, we combined and analyzed the single-cell transcriptomics (scRNA-seq) of zebrafish (with IBs) and medaka (without IBs). We identified the critical and tendon progenitor cell (ctsk+/sfrp1b+) with the ability of osteoblast differentiation by comparing the single cell data of zebrafish and medaka. And the ctsk+/sfrp1b+ cells might differentiate into osteoblast via bmp2b dependent on the TGF-beta pathway. On the other hand, ctsk+/sfrp1b+ cells were also present in the medaka, but them were loss of bmp2 activity. This study revealed the critical original tendon progenitor cells with an ability of osteoblast differentiation as well as the critical pathway and genes involved in tendon ossification and provided a hypothesis that the tenocytes also could differentiate into osteoblasts in fishes without IBs.

Keywords: tendon ossification, intermuscular bones, scRNA-seq, ctsk+/sfrp1b+ cells, bmp2

P18- EARLY LIFE PROGRAMMING AND MOLECULAR REGULATION OF THE STRESS RESPONSE IN GREATER AMBERJACK (*SERIOLA DUMERILI*) LARVAE

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SUMMARY

The greater amberjack (*Seriola dumerili*) is a relatively new aquaculture species of high economic interest. Yet, larval rearing protocols need to be optimized, to achieve higher larval survival rates, better growth, and lower variability in body size. To this end, the current study aimed at examining the effects of early life programming in the development of the stress response axis in greater amberjack larvae. To do so, larvae were exposed to two different water temperatures at early ontogeny: (i) at constant 24°C throughout the larval rearing period or (ii) at 20°C from embryos until the end of the autonomous feeding period and subsequently at 24°C until the end of the rearing period. Additionally, both temperature groups were further divided into subgroups based on the live feed provided: (a) only rotifers or (b) a combination of rotifers and copepods for 6 days starting at the first feeding. Samples of larvae were collected at specific developmental stages throughout the rearing period and analyzed for cortisol, cortisone and gene expression of various stress response regulating genes. During the autonomous feeding stage, no differences in the whole-body cortisol and cortisone concentrations, as well as in the mRNA expression of genes involved in the regulation of the stress response were observed between 20°C and 24°C. Flexion of the notochord was the developmental stage showing the most differences due to early temperature, as well as feeding. Finally, differences between stages due to development were also observed. Overall, it was shown that feeding and temperature affect the development of the stress response axis in the greater amberjack.

Keywords: development; early life programming; greater amberjack; larval rearing; stress response

P19- GENETIC ANALYSIS OF GROWTH TRAITS IN FUGU, *TAKIFUGU RUBRIPES*

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SUMMARY

In 2021, 50 families of tiger pufferfish (*Takifugu rubripes*) were produced and reared in common tanks (Tangshan, China). About 2100 juvenile fish were randomly selected, PIT tagged, and phenotyped for initial body weight (BW) and body length (BL). These fish were cultured successively in three different environments: outdoor pond (fresh food, 102 days), indoor tanks (formulated feed, 201 days), and sea cages (fresh food, 183 days). Initial and final BW and BL were recorded for each environment and growth rates were calculated. At harvest size (end of the sea cage period), 813 fin clips were collected from randomly selected fish for genotyping with a 20K SNP array. WGS data from the parents (22 dams and 23 sires) was available and used to impute the 20K genotypes up to 1.1 million SNPs in the whole population. Variance component and genome wide association analyses were performed on growth traits for each environment and genetic correlations between them were computed.

Medium to high heritabilities were estimated for growth traits, ranging from 0.20 to

0.57. A major QTL associated with growth in indoor tank and sea cages was detected on chromosome 22. Significant but moderate GxE was observed for growth in outdoor pond and both indoor tanks (0.61 ± 0.22 se) or sea cages (0.54 ± 0.18 se) and between indoor tanks and sea cages (0.64 ± 0.10 se). In summary, the genetics underpinning growth in pufferfish are complex and need to be carefully considered in the context of a breeding programme.

Keywords: *Takifugu rubripes*, GWAS, Growth, WGS

P20- EVOLUTIONARY DYNAMICS OF PARALOGOUS GENES IN OXIDATIVE PHOSPHORYLATION AND ONTOGENETIC REGULATION IN GILTHEAD SEABREAM (*Sparus aurata*)

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SUMMARY

This study delves into the crucial role of oxidative phosphorylation (OXPHOS) during the early developmental stages of the gilthead seabream (*Sparus aurata*) and the influence of the Teleost-Specific Whole Genome Duplication (tsWGD). OXPHOS is the primary mechanism responsible for over 90% of cellular ATP production, orchestrated by five multiprotein complexes encoded by both mitochondrial and nuclear genomes. The tsWGD, provides a unique context to explore how the necessities of OXPHOS shape developmental outcomes. The focus is on the highly dynamic phases of early seabream ontogeny, characterized by significant changes in morphology, physiology, metabolism, and behavior. Utilizing transcriptome and whole genome sequencing, OXPHOS genes and their paralogs are identified within the seabream genome. The subsequent analysis reveals diverse expression patterns among paralogs, including dosage balance, stage-specific expression, and differential expression across developmental stages. Furthermore, integration with whole genome sequencing data uncovers subtle variations in the number and type of mutations among OXPHOS paralogs. The ratio of non-synonymous to synonymous substitutions when comparing the CDS variants revealed Purifying and Neutral Selection in action, safeguarding protein structural integrity and/or function. The findings suggest that the retention of OXPHOS paralogs in the seabream genome, facilitated by the tsWGD, contributes to ontogenetic plasticity through regulatory sub-functionalization. This study provides insights into the evolutionary mechanisms shaping the intricate relationship between whole genome duplication, OXPHOS evolution, and the demands of development in a highly adaptive organism during its early stages.

Keywords: Evolution, Ontogeny, Gilthead seabream (*Sparus aurata*), Oxidative phosphorylation (OXPHOS), Teleost-Specific Whole Genome Duplication (tsWGD)

P21- GENETIC RESILIENCE IN THE COMMON COCKLE (*Cerastoderma edule*): CONTRASTING NATURAL SELECTION AND SELECTIVE BREEDING FOR MARTEILIOSIS RESISTANCE

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SUMMARY

The infection with the protistan *Marteilia cochillia* emerged in Galician (NW Spain) coast in 2012, causing cockle *Cerastoderma edule* fishery collapse in the Ria de Arousa estuary. Eventually, marteiliosis declined due to increased cockle resistance through natural selection. Previous studies identified 28 SNPs associated with resilience in cockles in this estuary, and a selective breeding program was developed, succeeding in increasing marteiliosis resilience in the 2nd generation (F2). Our study aimed to validate the resilience marker SNPs in the selective bred cockle stock compared to naturally resilient cockles. Three broodstocks were established with cockles from: a naïve population in Ria de Muros-Noia (NO), a marteiliosis-affected bed in Vilanova de Arousa (VA, Ría de Arousa), and the F2 of the breeding program. Seed batches from each broodstock were hatchery-produced (NO, VA and F3) and field-challenged in two marteiliosis-affected areas. Results revealed high marteiliosis prevalence and cockle mortality in the NO stock, whereas much lower in VA and F3. Cockles were genotyped before and after challenge with Sequenom MassARRAY technology, using 47 SNPs able to differentiate naturally resilient from naïve cockles. Genetic diversity (He: 0.263-0.327) was lower in F3 cockles, which exhibited the lowest effective population size (Ne: F3 =25, NO=77 and VA=87). Six markers consistently increased genetic diversity post-challenge. Despite global F_{ST} was low (F_{ST}=0.025), 18 markers displayed significant genetic differentiation between the naïve, naturally selected and the selective bred stocks (0.018-0.103). This information is being evaluated for cockle fisheries management in Galicia.

Keywords: Cerastoderma edule, Resistance, SNPs, Marteiliosis, Breeding program.

P23- APPLYING A CHROMOSOME-SCALE ASSEMBLY TO EXPLORE POPULATION STRUCTURE, METAGENOMICS AND EVOLUTION IN THE BLUE MUSSEL (MYTILUS EDULIS)

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SUMMARY

The blue mussel is commonly described as the *Mytilus* species complex, encompassing at least three putative species: *M. edulis, M. galloprovincialis* and *M. trossolus*. These three species occur on both sides of the Atlantic and hybridize in nature, and both *M. edulis* and *M. galloprovincialis* are important aquaculture species. They are also invasive species in many parts of the world. We report on the first chromosome-level assembly of the *Mytilus* species complex. Using a combination of PacBio sequencing and Dovetail's Omni-C technology to generate an assembly with 14 long scaffolds containing 94% of the predicted length of the *M. edulis* genome. Using GBS and shotgun sequencing, we also sequenced 3 North American populations of *Mytilus* to characterize single-nucleotide as well as structural variance. A combination of AB-*Initio* annotation and Isoseq transcriptomic analysis identified 65,505 gene models. This high-quality genome for *M. edulis* provides a platform to develop tools that can be used in breeding, molecular ecology and evolution to address questions of both commercial and environmental perspectives. This includes studies examining the significantly high levels of gene Presence Absence Variation known to exist in mussels, metagenomic analyses and our own orthology analyses studying gene family expansions and evolutionary adaptations in the *Mytilus* species complex.

Keywords: Mussel, genomics, orthology, evolution

P24- A HEATWAVE EVENT AFTER HATCHING ALTERS DNA METHYLATION PROFILE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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SUMMARY

Several studies have characterized the potential effects of global warming, but little is known about the effects of extreme heatwave events and their related molecular mechanisms. DNA methylation is influenced by environmental signals, and links genome and phenotype. The survival, skeletal development, DNA methylation profile and the expression of particular genes in key tissues of rainbow trout juveniles exposed to a thermal stress (mimicking a naturally occurring heatwave event, reaching 16 or 20 °C in the climax (T16 and T20 groups, respectively)) were evaluated and compared with control specimens reared at 12 °C. Survival was lower in T20 juveniles, while both T16 and T20 specimens showed an increased incidence of jaw deformities (4% in Control fish *versus* 20 and 12 % in T16 and T20 juveniles, respectively). DNA samples from Control and T20 juveniles were submitted to an Enzymatic Methyl-sequencing (EM-Seq) procedure. 0,31 % of CpGs from Control and T20 juveniles were differentially methylated (DM). Most DM CpGs were located at intergenic (50-53 %) and intronic (32-33 %) regions, but a 11-13 % were found at promoter regions. Gene expression (qPCR) analysis of most promising genes (located at less than 2000 bp distance from the DM CpGs) confirmed lower gene expression in T20 specimens, as expected by DNA methylation analysis. Present study not only unveils the potential effects of the extreme events under a climate change scenario, but also identifies potential adaptive mechanisms (DNA methylation) in future farmed fish. Work funded by PID2021-127782OA-I00 and PIE 202230/166 projects, and RYC2018-025337-I grant.

Keywords: epigenetics, thermal stress, aquaculture, early development, skeletogenesis

P25- DUE TO THEIR IMPROVED IMMUNITY, DISEASE RESISTANT COMMON CARP FISH ARE ALSO LESS INFECTIVE

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SUMMARY

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. When infected, resistant fish restrict viral replication and survive, while susceptible fish cannot and succumb to the disease. This resistance mechanism involves improved immunity. In this study, experiments using infection-by-cohabitation tested the infectivity of disease-resistant and susceptible fish and how differences in resistance and infectivity affect mortality and disease spread. Disease resistant and susceptible fish played roles of shedders (infecting) and cohabitants (infected) in all four type-role combinations. Mortalities were highest in susceptible cohabitants infected by susceptible shedders and lowest in resistant cohabitants infected by resistant shedders. Surprisingly, fewer mortalities were found in susceptible cohabitants infected by resistant shedders compared to resistant cohabitants infected by susceptible shedders, indicating that who the shedder is does matter. Accordingly, spleen viral loads in resistant cohabitants infected by resistant shedders were lower than in those infected by susceptible shedders. Finally, virus levels in water of tanks holding susceptible shedders were higher than in tanks holding resistant shedders. Taken together, we empirically and clearly demonstrated that since disease resistant fish control better the virus replication they release less virus particles into the environment and hence, infect other fish less than susceptible fish. This study demonstrates that aquaculture production will benefit twice from incorporating resistant fish, by reducing both mortalities and disease spread.

Keywords: Cyprinus carpio, Cyprinid herpes virus type 3, Koi herpes virus, Disease resistance, Infectivity

P26- A NEW SEX DETERMINING GENE IDENTIFIED IN STRIPED BASS

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SUMMARY

Crosses between the marine fish striped bass (*Morone saxatilis*) and the freshwater fish white bass (*Morone chrysops*) are practiced for producing the aquaculture fish hybrid striped bass. Interspecific hybrids are used in aquaculture for their improved performance in key traits, including for producing sterile or mono-sex populations. Interspecific hybrids also provide opportunities to study the evolutionary conservation of sex-determination systems and how parental mechanisms are combined to determine sex in hybrid progeny. Using genotyping-by-sequencing, we identified a single SEX QTL in striped bass. By sequencing candidate genes in this region, we identified a deletion in the sequence of *Cyp19a*, a gene coding for gonadal aromatase. We verified this sex-associated variant in males and females from different populations and found that males were homozygotes and females were heterozygotes, indicating a ZZ/ZW sex determination mechanism. We then cloned and expressed the different aromatase alleles and tested for a difference in estradiol production, under the model that the male allele is not producing estradiol, hence driving the development of testes. We further tested for the conservation of this sex-determination mechanism and found that the closely related white bass had evolved a different mechanism. How these two mechanisms combine to determine sex in the hybrid is being studied. Taken together, we identified a new sex-determination gene in the striped bass that works in a ZZ/ZW mechanism. Supported also by the studies in the related European sea bass, it seems that sex-determination mechanisms in moronidae are variable.

Keywords: Sex determination, ZZ/ZW mechanism, Morone saxatilis, Morone chrysops, Hybrid striped bass

P27- GENETIC ANALYSIS AND DEVELOPMENT OF SEX-SPECIFIC MARKERS IN POPULATIONS OF MEGALOBRAMA AMBLYCEPHALA BASED ON WHOLE-GENOME RE-SEQUENCING

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SUMMARY

Blunt snout bream is an important freshwater aquaculture species in China. However, due to the lack of conservation of germplasm resources during long-term aquaculture, the populations of blunt snout bream have exhibited adverse trends such as uneven individual sizes, reduced growth rates, and early sexual maturation, hindering the further expansion of the industry. Understanding the genetic structure and genetic diversity of the blunt snout bream population and cultivating monosex populations through sex control technology will facilitate the rational utilization and conservation of its genetic resources. In this study, we used re-sequencing technology to construct a genomic genetic variation database of three natural and three genetically improved populations of blunt snout bream. Based on the SNP variations, we carried out an analysis of population genetic structure and genetic diversity. The results revealed relatively low levels of polymorphism in the six populations, with moderate to high genetic differentiation between the genetically improved populations and their source populations. Selective sweep analysis revealed olfactory signal transduction, immune response, and metabolism-related pathways associated with growth and tolerance to low oxygen, providing insights into the genetic basis of these important economic traits. Furthermore, three male-specific molecular markers for the blunt snout bream were developed through comparative analysis of female and male genomes, and one of these markers was successfully applied to Megalobrama pellegrini, a species within the same genus. The results will provide support for the conservation of genetic resources and sex-controlled breeding of the blunt snout bream.

Keywords: *Megalobrama amblycephala*, genome re-sequencing, genetic diversity, male-specific molecular markers

P28- GENE EXPRESSION PROFILING OF SIX6 AND BCL6A IN LARVA AND JUVENILE STAGES IN EUROPEAN SEABASS (*DICENTRARCHUS LABRAX* L.)

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SUMMARY

The genomic study of the domestication process in the European seabass (*Dicentrarchus labrax*), revealed genomic regions with markedly different allele frequencies between wild and farmed populations. Strong candidate genes that were detected in these regions involve the *six6* and *bcl6a* genes known to be associated with key commercial traits such as maturation and growth rate, which may be targeted by breeding programs applied in the fish farming sector. The *six6* gene has an evolutionarily conserved role in maturation and has already been studied in other teleost species, such as Atlantic salmon (*Salmo salar*). Likewise, the *bcl6a* gene was shown, through knockout experiments in zebrafish (*Danio rerio*), to be involved in the growth and maturation of larvae. In this study, we investigated the gene expression profiles of *six6a* and *bcl6a* genes at two developmental stages (larvae and juveniles) that were grown under the same conditions. By investigating the association of the SNPs genotypes of the genes under study along with their expression pattern, we aimed to elucidate the effect of *six6* and *bcl6a* on the developmental process of European seabass and their potential evolutionary conserved function.

This study was conducted under the project "SystEms Biology Modelling of Key LIFe History Traits for Sustainable Aquaculture Production in the Mediterranean Region'', funded by H.F.R.I.. We would like to thank Assoc. Prof. Michalis Aivaliotis (MA) and Stefania Maniatsi, PhD from Functional Proteomics and Systems Biology Research Group at AUTh (FunPATh; headed by Assoc. Prof. MA), for hosting the qPCR experiments performed using the MIC qPCR Cycler from Bio Molecular Systems (kindly provided by Biodynamics S.A., Athens, Greece) and for their help in using the MIC qPCR Analysis Software.

Keywords: gene expression, developmental stages, European seabass, *six6*, *bcl6a*

P29- USING LONG-READ SEQUENCING WITH OXFORD NANOPORE TO ENABLE GENOMIC AND EPIGENOMIC PROFILING OF LIVESTOCK FISH

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SUMMARY

Long-read nanopore sequencing data enables construction of more complete and contiguous genome assembles than short-read data, and, at the same time, can be used to reveal genome-wide epigenetic methylation signatures. Thus, it represents a valuable tool for advancing our understanding of evolutionary and adaptive mechanisms of the livestock fish. In this study, raw long-read data (N50 read length = 13.1 kb, total = 30.36 Gb) from sea bream were processed using Flye to generate, in 18 h, an assembly containing 849 Mb of sequence with an N50 fragment length of 6 Mb. We also benchmarked the performance of long-read tools and resources identifying methylation signatures. To do this, we tested the effect of training models configurations (FAST/HAC/SUP), sequencing depth, and the performances of three different software (Guppy, Bonito, and F5C) on distinct evaluation criteria (execution time, CpG dinucleotides detection, and genomic locations). The FAST model does not provide extra information on methylation than HAC and SUP, independently of the used tool and the sequencing depth. SUP model utilization supposes a remarkable increase of execution/computation, but also an increase of the read quality. The three models map the same CGs, and are linearly correlated. The percentage of mapped CpG increases linearly from 3.4X to 20X, and results in 20X include >95% of 34X results, arising 20X as a minimum depth necessary for this type of procedures. Guppy arise as the better time-quality tool for methylation detection. Overall, a new pipeline for individual assembly and methylation detection from long-read data was designed.

Keywords: Long-read sequencing, Nanopore sequencing, genome assembly, methylation, bioinformatics tools benchmarking.

P30- THE EXPANSION OF SIRTUIN GENE FAMILY IN GILTHEAD SEA BREAM *(Sparus aurata)*. PHYLOGENETIC, SYNTENIC AND FUNCTIONAL INSIGHTS ACROSS THE VERTEBRATE/FISH LINEAGE

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SUMMARY

The seven mammalian sirtuin (SIRTs) counterparts have arisen as key components of diverse physiological processes. However, many questions related to their evolutionary origin and physiological significance remain largely unknown in vertebrate species. In sea bream, extensive homology-searches against the assembled genomic references widened this repertoire up to ten *sirt* paralogs, including three copies of *sirt3* (*sirt3.1a*, *sirt3.1b*, *sirt3.2*) and two of *sirt5* (*sirt5a*, *sirt5b*). A combined approach of phylogenetics, synteny, and gene expression profiling across tissues (screening of 11-tissues) and developmental stages (60 dph-3 years; skeletal muscle/liver) was addressed. Phylogenetics and synteny analyses revealed that the novel copy of *sirt5b*, neighboring *igfbp1b/igfbp3b* paralogs, was the result of a teleost-specific retention after the 3R whole genome duplication (WGD). The two copies of sirt3 (*sirt3.1, sirt3.2*) were retained in aquatic organisms, but not in terrestrial animals after the 2R WGD. Besides, the *sirt3.1* phylogenetic branch revealed a tandem-duplicated synteny block (*psmd13-sirt3.1a/b-drd4-cdhr5-ctsd*) in some fish, including sea bream, although it is unclear if this event was due to retention after 3R WGD or promoted by transposable elements. Gene expression revealed that *sirt3.*1 was mainly expressed in liver and muscle tissues, whereas *sirt3.2* was highly expressed in immune-relevant tissues. Both in liver and skeletal muscle, the *sirt3.1* expression levels increased through development, while the opposite was found for *sirt3.2* and *sirt5b*. Likewise, the *sirt5b*/*igfbp1b*/*igfbp3b* syntenic block was silenced through development in skeletal muscle. This study offers novel insights into the evolutionary patterns of Sirts, reinforcing the importance of gene duplication landscape in sea bream.

Keywords: Aquaculture, gilthead sea bream, sirtuin, phylogenomics, synteny, gene duplications, tissue-specificity, neo-functionalization, adaptive plasticity

P31- DIFFERENTIAL EXPRESSION OF MICRO RNAS (miRNAs) IN GONADS OF GILTHEAD SEABREAM *SPARUS AURATA*

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SUMMARY

MicroRNAs (miRNAs) play a crucial role in regulating reproduction-related genes in various organisms, including teleosts. They regulate processes such as germ cell differentiation, gametogenesis, steroidogenesis, and apoptosis. 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. As a result, some individuals have bisexual gonads. The present study aims to explore the role of miRNAs in the bisexual testes and ovaries of the gilthead seabream. To achieve this aim, six fish (three females and three males) aged six years old were sacrificed in February 2019. The two different parts of the bisexual testis (mature male, M and immature female, fM), as well as ovaries of mature females (F) were excised and preserved for histology and RNA extraction. Subsequently, sncRNA libraries were generated, sequenced, and differential expression analysis was conducted. After quality and adaptor trimming, the average number of reads per sample was 13 million. The read length distribution displayed two main peaks: one at 21-24 nt, corresponding to miRNAs, and one at 25-30 nt, corresponding to piwi-interacting RNAs (piRNAs). As expected, expressed miRNAs formed three distinct groups, with mature females being closer to immature females. The number of differentially expressed miRNAs among groups are shown in a Venn diagram and putative miRNA targets are explored.

Keywords: gilthead seabream, miRNAs, differential expression, gonads, reproduction

P32- MOLECULAR IDENTIFICATION OF BACTERIAL PATHOGENS ASSOCIATED WITH DISEASES IN AQUACULTURE FARMS IN THE PHILIPPINES

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SUMMARY

Aquaculture is a vital sector in the Philippines, contributing significantly to both food security and economic livelihoods. However, the intensification of aquaculture has led to an increase in the occurrence of bacterial diseases, posing a significant threat to the industry's sustainability. This study aimed to identify pathogenic bacteria associated with disease and/or mortality outbreaks in various aquaculture farms in the country. Different fish commodities, such as Nile tilapia (*Oreochromis niloticus*, n = 83), African catfish (*Clarias gariepinus*, n = 17), and milkfish (*Chanos chanos,* n = 21), were collected from farms and hatcheries all over the Philippines and were identified by molecular methods such as *16S rRNA* and *rpoD* gene sequencing, and PCR using species-specific gene markers. The diagnostic sampling for farmed Nile tilapia revealed the presence of *Aeromonas veronii* (19.28%), *Streptococcus agalactiae* (14.46%), *Edwardsiella tarda* (9.64%) and *A. hydrophila* (3.61%). Additionally, both *A. veronii* and *A. hydrophila* were detected in African catfish with a prevalence of 17.65% each. In contrast, bacterial isolates from milkfish exhibited the presence of *Vibrio harveyi* (47.62%) and *V. alginolyticus* (9.52%). The findings of this study provide baseline information for future investigations concerning bacterial diseases in aquaculture and are essential in the development of control programs and therapeutic strategies against disease outbreaks which currently is affecting aquaculture industry in the Philippines.

Keywords: molecular identification, polymerase chain reaction (PCR), aquaculture, pathogenic bacteria

P33- UNDERSTANDING THE GENETIC BASIS OF PIGMENTATION ANOMALIES IN FLATFISH

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SUMMARY

Pigmentation abnormalities are a common problem in flatfish aquaculture, which significantly reduces the commercial value of affected fish. In turbot, these abnormalities mainly manifest as pseudoalbinism (a partial or total absence of dark pigmentation on the ocular side) or ambicolouration (total darkness on the blind side). The cause of the relatively high incidence of pigmentation anomalies is unknown but it is likely due to complex interactions between genetic and environmental factors. To elucidate the genetic basis of altered pigmentation, we estimated the heritability of this trait and conducted genome-wide association studies (GWAS) on turbot with both normal and altered pigmentation patterns. A cohort of 783 individuals from 10 families underwent genotyping using a low-density SNP panel, while their parents underwent whole-genome sequencing. Based on genotyped data, the heritability of this trait was found to be 0.65±0.09. GWAS identified two possible candidate SNPs on chromosomes 10 and 15, respectively, which explain, however, a low proportion of the phenotypic variance. This study indicates that normal pigmentation can be included as a selection target in selective breeding programmes. Additional analyses, which involve imputing low-dense genotypes to whole-genome sequences of the parental turbot are expected to provide valuable information and improve the accuracy of the results.

Keywords: GWAS, heritability, pigmentation abnormalities, selective breeding, turbot.

P34- THE EFFECT OF DIETARY *RHODOTORULA MUCILAGINOSA* ON LIVER ANTIOXIDANT GENE EXPRESSION OF *SPARUS AURATA*

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SUMMARY

Fish in aquaculture are exposed to various stressors, therefore they are susceptible to oxidative stress. Oxidative stress is caused by a disequilibrium between the production of Reactive Oxygen Species, like hydrogen peroxide and lipid peroxides, and the ability of an organism to detoxify them. The use of yeasts as fish feed additives in aquaculture can be an efficient tool to promote fish antioxidant status. The goal of the study was to evaluate the effects of the inclusion of *Rhodotorula mucilaginosa*, on the antioxidant gene expression of *Sparus aurata*. One hundred twenty (120) *Sparus aurata* juveniles were acclimatized to laboratory conditions, and were randomly placed into 12 tanks. Four experimental diets (0%, 1%, 2% and 3% inclusion) containing lyophilized *R. mucilaginosa* strain ACA-DC 5340 obtained by ACA-DC collection of Agricultural University of Athens. Each dietary treatment was triplicated. At the end of the experiment, the liver of six (6) fish from each treatment (24 fish in total) was dissected and stored at -80ºC. Total RNA was extracted and cDNA was prepared. Genes of interest (*gpx*, *gr*, *cat* and *sod*) were amplified and relative gene expression was studied. The results indicated that the antioxidant equilibrium is modulated by the use of dietary supplementation in aquaculture fish.

Keywords: *Sparus aurata*, *Rhodotorula mucilaginosa*, feed supplementation, gene expression, antioxidant system

P35- GRINNAQUA: GREEN INNOVATION STRATEGIES FOR ANIMAL HEALTH MANAGEMENT: TOWARDS SUSTAINABLE AQUACULTURE

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SUMMARY

In order to strengthen CIIMAR's research, amplify its innovative capabilities and secure its position as a dominant force within the aquaculture industry, GRINNAQUA is strategically aligned with the goals of the Farm-to-Fork Strategy. The partnership with well-known institutes in European aquaculture research, with extensive knowledge in relevant fields, will boost CIIMAR´s competitiveness. The Spanish institution INIA-CSIC is skilled at vaccination procedures and has a deep understanding of fish acquired immunity. The University of Bergen (Norway) is highly competent in the area of animal health, while the Roslin Institute (UEDIN, UK) has invaluable knowledge in the area of genetics and animal breeding.

To enhance the level of excellence of CIIMAR, the collaborating partners are putting into place specialized training sessions and immersive Summer schools.

A collaborative effort that makes use of the institutions' combined scientific and technical resources is at the heart of the research agenda. The goal of this coordinated effort is to address urgent problems associated with economically significant outbreaks in the aquaculture industry. The project intends to bring insight into the preventive potential of functional feeds for shielding rainbow trout against the haemorrhagic septicaemia virus and the infestation of Atlantic salmon with sea lice.

In essence, GRINNAQUA project plays a crucial part in strengthening CIIMAR's capacity for innovation, establishing the organization as a leader in bringing about the blue revolution in the aquaculture industry. This partnership is likely to be long lasting and to create synergies that significantly reinforce a more resilient and sustainable aquaculture landscape throughout Europe.

Keywords: Genetics, Vaccination strategies, Animal welfare, Collaborative project, functional feeds

P37- GENETIC IDENTIFICATION OF STURGEON POPULATIONS FROM UKRAINE USING DNA MARKERS

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SUMMARY

It was investigated the genetic features of sturgeons caught in the territorial waters of Ukraine.

The results of the researches made it possible to determine the peculiarities of the genetic structure and to conduct interspecific comparison and genetic identification of the studied sturgeon representatives using DNA markers.

Keywords: Sturgeons, genetic identification, DNA markers.

P38- MOLECULAR CHARACTERIZATION OF BREEDERS OF *Solea sengalensis*

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SUMMARY

The flatfish senegalese sole (*Solea senegalensis*, Kaup 1858) farming faces a bottleneck since males raised in captivity (F1) do not exhibit the reproductive behaviours observed in wild male breeders, especially prespawning chase or paired synchronized spawning, which has been pointed out to be the primary cause of reproductive failure. In addition, they show poor gonadal development and gamete production.

The main difference between rearing and natural conditions is the higher temperature and the possible lack of thermal variability at which F1 male development takes place. When individuals were sorted by sex, the transcriptome and metilome analyses of adult males and females, both mature and immature, and of wild or F1 farm origin, revealed significant differences in gene expression.

Related to data of transcriptomes, in a principal component analysis (PCA), female samples showed a strong clustering indicating great similarity, while males showed two groups differentiated by their origin, wild or F1. In addition, an analysis of CpG region (or CpG islands) methylation levels identified was performed to assess the differences in methylation between the groups considered when comparing them to each other, contrasting males/females and wild/domestic groups. Significant differences were observed in all comparisons, highlighting higher levels of methylation in females than males, and in domestic groups than in wild groups.

Funding grants: Spanish FEDER-P-20-00938; Spanish FEDER- CCMM-00014

Keywords: Senegalese sole, flatfish, transcriptomics, methylation, reproduction

P39- A SINGLE HEATWAVE EVENT IS ABLE TO DISRUPT SPERMATOGENESIS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) BREEDERS

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SUMMARY

Expansion of global aquaculture will most probably be impacted by climate change. The present study is focused on recreating more realistic models of climate change, particularly considering heatwave phenomena's, to decipher their effects on fish spermatogenesis. Two experimental groups were established: i) trout males subjected to a heatwave event (Hw group): with a progressive water temperature increase (1 °C day-1), until reaching 20 °C, maintained for eight days and finally the initial temperature (14 °C) being restored; and ii) Control fish (Ctrl), constantly maintained at 14 °C. Plasma cortisol and testosterone, sperm motility at different maturation times, gonad germ cell proliferation and gonad transcriptomic profile were evaluated in both groups. Increased cortisol plasma levels and lower sperm motility were found in the Hw group compared to Ctrl males. Germ cell proliferation in gonads was disrupted, with reduced abundance of spermatocyte I and II in Hw males. Consequently, we decided to investigate the molecular alterations that trigger this spermatogenesis failure in Hw males. RNA-Seq analysis of testes showed as in 57 and 423 genes were up- and down-regulated in Hw males (fold change ≥ 1.5; p adjust. value < 0.01). GO analysis showed that apoptosis KEGG pathway was positively overrepresented while sperm axoneme assembly and other biological processes related to sperm functionality were negatively overrepresented. Present results suggest that heatwave events threaten reproductive performance, and that not only predicted scenarios of global warming will compromise aquaculture growth. Work funded by REPHEAT (PID2021-127782OA-I00) project.

Keywords: thermal stress, aquaculture, reproduction, germ cell, transcriptomics.

P40- A HIGH-QUALITY GENOME ASSEMBLY OF THE MEDITERRANEAN MUSSEL (*Mytylus galloprovincialis*) PROVIDES INSIGHTS INTO MUSSEL SEX DETERMINATION

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SUMMARY

Sex determination (SD) systems regulate the sexual development of individuals, have broad implications in the phenotypes of many aquaculture species via sexual dimorphism. The Mediterranean mussel (*Mytilus galloprovincialis*) is one of the most important species in European aquaculture and is found in coastal regions worldwide. Bivalves present highly diverse SD systems, and they remain poorly understood in most species, including the Mediterranean mussel. Studying SD is crucial for understanding the differences between males and females in traits of interest, such as colour, length and size. For this purpose, 25 mussel families were used in a massal cross (5 males x 5 females). Around 1000 offspring were obtained, sexed by histology and assigned to their families by microsatellites. Measurements were taken for colour, size, and length, and logistic regression was used to estimate global, maternal, and paternal heritabilities for sex. Moreover, a new highly contiguous genome assembly of *M. galloprovincialis* was constructed using long-read sequencing, and used to identify SNPs and allelic frequencies showing differences between males and females. For this, reads obtained from whole genome resequencing of 10 males and 10 females were filtered and aligned against the new genome assembly. The results indicated that maternal effects were mainly responsible for SD in this species. Additionally, genomic region showing high F_{ST} values ($p<0.001$) between males and females allowed us to identify candidate master SD genes for the Mediterranean mussel. These findings provide important molecular tools for further investigation into SD of mussels, with potential application in the aquaculture industry.

Keywords: Mediterranean mussel, heritability, sex determination, candidate master genes

P41- SINGLE-CELL TRANSCRIPTOME AND EPIGENETIC PROFILING OF THE PITUITARY GLAND PROVIDE INSIGHTS INTO TELEOST PUBERTY

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SUMMARY

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, in industrial-scale sea cages their application is limited. As puberty is largely affected by external stimuli, we hypothesize that transcriptional and epigenetic signals in key tissues, such as the pituitary gland, can shed light into the fundamental biological networks that regulate puberty. Thus, we performed a pilot study using Nile tilapia (*Oreochromis niloticus*) as a model organism for reproductive endocrinology. Fish were exposed to continuous or ambient light for 4 months and their morphometric characteristics were recorded. To capture the in-depth biological mechanisms that underlie puberty, we used state-of-the-art single-cell multiome (RNA-seq and ATAC-seq) profiling. We identified major cell types with distinct gene expression (Figure 1) and chromatin accessibility profiles. By performing differential expression (DE) and accessibility (DA) analysis, we were able to identify key cell types of the pituitary gland and biological markers that may play an important role in the onset of puberty in fish. These results will be utilized in project EPICOD for the optimization of single-cell analysis and cell-type annotation in Atlantic cod (*Gadus morhua*) and European seabass (*Dicentrarchus labrax*) and offer a baseline for interspecies comparisons among commercially valuable fish species.

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

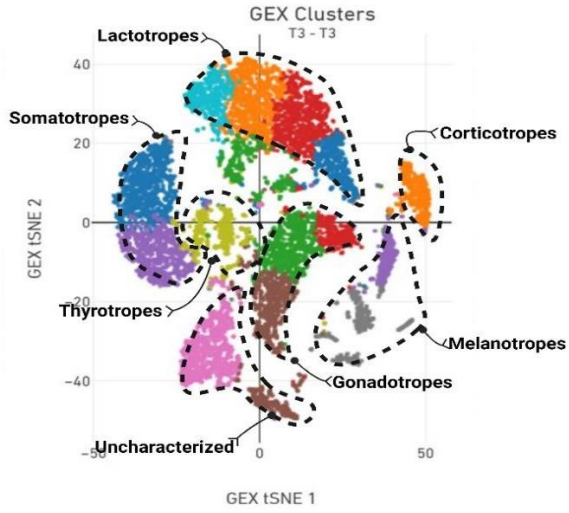

Figure 1. tSNE plot of gene expression visualizing cluster assignment of pituitary cells.

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P42- SKMER – GENETIC DIVERSITY METRICS FROM GENOME SKIMS FOR BROODSTOCK SELECTION

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SUMMARY

In marine aquaculture, key species such as salmon, oysters, tilapia, and shrimps have their genomes sequenced, often to chromosome-level detail. However, many high-value species still lack publicly available, high-quality reference genomes, which limits their genomic resources. Genome skimming with low-coverage whole-genome sequencing (lcWGS) can speed up broodstock selection, helping to ensure the best population fitness for aquaculture by using insights into genetic diversity. Skmer is a bioinformatics tool that leverages WGS data to estimate genome distances without needing assembly or alignment and provides accurate estimates of important population genomics metrics like F_{ST}, heterozygosity, and population structure. Skmer is viable alternative notably for broodstock selection in aquatic species with complex genomic features and could help small local aquaculture endeavours.

Keywords: Genome-skims, genome reference-free, FST, heterozygosity, lcWGS

P43- ASSESSING THE IMPACT OF INSECT MEAL ON THE INTESTINAL BACTERIAL MICROBIOTA OF THE BALTIC SHRIMP *PALAEMON ADSPERSUS*

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SUMMARY

The present study aims to characterize for the first time the structure and composition of bacterial communities in the intestine of Baltic shrimp, *Palaemon adspersus* (Rathke 1837), apropos the dietary regime. In this context, insect meals derived from the larvae of yellow mealworm (*Tenebrio molitor*), black soldier fly (*Hermetia illucens*), and common housefly (*Musca domestica*) partially substituted fishmeal as the main protein source in the diet of *P. adspersus*. The intestinal bacterial microbiota was profiled by high-throughput sequencing of the 16S rRNA gene V3-V4 region. The results revealed that the intestinal bacterial communities were dominated by Proteobacteria, while the dietary inclusion of insect meals elevated the relative abundance of Firmicutes potentially due to alterations in diet composition such as the carbohydrate source. Among the insect meals studied herein, *H. illucens* seems to converge, to a higher degree, with the diversity patterns of the control group, while simultaneously favored the Firmicutes to Bacteroidetes ratio, an indicator of gastrointestinal health. The latter substitute may promote microbial balance contributing, thus, to the apparent growth amelioration of *P. adspersus*. In contrast, the fishmeal substitution with *T. molitor* altered the bacterial diversity and evenness, which enabled the appearance of opportunistic and/or potentially pathogenic bacteria (e.g. *Marinomonas* spp.) suggesting a shift to potential disruption of the bacterial communities metabolic traits. Nevertheless, insect meals promoted the enhancement of several beneficial microbes. The present findings demonstrate that the source of the insect meal may differently affect the shaping of intestinal bacterial communities.

Keywords: microbiota, gut dysbiosis, 16S rRNA, insect meal, sustainable aquaculture

P44- STUDY ON THE DEVELOPMENTAL CHARACTERISTICS AND MOLECULAR REGULATORY MECHANISMS OF DIFFERENT TYPES OF INTERMUSCULAR BONE IN COILIA NASUS

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SUMMARY

Intermuscular bones (IBs) are small needle-shaped bones that ossify from the tendons in the fish myosepta. The existence of IBs has seriously affected the consumption and processing of many farmed fish. The adverse effects of IBs on fish quality improvement, breeding efficiency, and industrial development have gradually made IBs a hot topic for discussion and research in the fields of fish development and genetics. Early studies found that fish IBs not only have complex morphological characteristics but also have different distribution characteristics in different evolutionary branches of fish. For example, cypriniform fish generally have significant numbers of epineural and epipleural bones, while perciform fish such as tilapia, perch, and mandarin fish only have typical epicentral bones, and some herring fish such as Coilia nasus have all 5 types of IBs. Previously, we identified the conserved regulatory gene runx2b for the epineural and epipleural bones and used gene editing technology to create new germplasms such as zebrafish and bream without IBs. However, it is unclear whether the five types of IBs have the same developmental characteristics and conserved regulatory factors. Considering that Coilia nasus has evolved all five known types of IBs in fish and has become a model species for studying the molecular regulatory mechanisms of multiple types of IBs in fish, we carried out studies on the histological and genetic basis of IB formation in Coilia nasus. We explored the development rules, tissue structure changes, and cell differentiation characteristics of different types of IBs. Multi-omics analysis technology was used to carry out tissue traceability analysis and reveal the origins of different types of IBs. We also identified specific and conserved regulatory genes for different types of IBs and verified their expression patterns and functions. The research results have clarified the similarities and differences in the developmental characteristics and molecular regulation of different types of IBs in fish, promoted an in-depth analysis of the formation mechanism of IBs, and provided theoretical support for the genetic improvement of economically important fish with different types of IBs.

Keywords: Intermuscular bone, Developmental characteristics, Cell differentiation, Molecular regulatory, *Coilia nasus*

P46- EXPLORING GENETIC DIVERSITY AND HYBRIDISATION PATTERNS OF MUSSELS IN NORTHERN SCOTLAND

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SUMMARY

Understanding genetic diversity and gene flow within and between populations of mussels grown in aquaculture informs population fitness and contributes towards sector sustainability. Shetland, in the north of Scotland, is responsible for >50% 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 in the north of Scotland. DNA was isolated from samples taken from four populations, each containing 30 animals: one in the northeast of Scotland (Cromarty Firth); the 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 developed by Jenny Nascimento-Shulze et al 2023. To analyse subspecies hybridisation, we also included reference samples of *Mytilus* subspecies: *M. galloprovincialis, M. edulis* and *M. trossulus.* The initial analysis reveals that the population in Cromarty appears to be a predominantly *M. edulis* background. 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. 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

P47- BROODSTOCK MANAGEMENT INFLUENCES THE METABOLIC STATUS, GENE EXPRESSION AND EPIGENETIC GENE REGULATION IN PROGENY

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SUMMARY

In the Atlantic salmon farming sector, adjusting the spawning season of female broodstock is a widespread strategy to ensure a steady supply of offspring all year-round. Environmental factors such as water temperature, light exposure, and feeding schedules are manipulated to optimize reproductive timing. Therefore, gaining a deeper understanding of the biological mechanisms influenced by these alterations is crucial for improving the growth performance of the offspring, as they are important as the production fish in aquaculture.

In this study, we examined in detail four different spawning seasons achieved through two different production methods from commercial production in Norway: recirculating aquaculture systems (RAS) and sea-pen-based broodstock. Alongside the normal spawning season in sea-pens in November, we analysed three adjusted seasons: an off-season (five-month advance in RAS), an early season (two-month advance in sea pens), and a late season (two-month delay in sea pens). Previous findings have demonstrated that the altered spawning seasons significantly affect both the nutritional status of broodstock and offspring, thereby impacting nutrient transfer and offspring development. Here, we show significant changes in metabolic profiling, the patterns of gene expression (RNA-seq method) and DNA methylation (RRBS method) due to the altered spawning seasons. Offspring from RAS-based off- season spawning exhibited reduced larval weights and displayed changes in 1C metabolism genes and lipid-mediated regulations. Both early and late seasons impacted cellular processes, particularly cell cycle regulation. Here we show metabolic and epigenetic consequences in offspring due to broodstock management practices. We suggest studying the critical stages of broodstock development during which they are responsive and sensitive to management practices, thus impacting the above findings in offspring phenotype.

Keywords: Broodstock, Spawning season, Intergenerational epigenetics, Metabolomics

P48- GENE EXPRESSION PROFILE OF THE COCKLE *CERASTODERMA EDULE* IN RESPONSE TO TREMATODE INFECTION

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SUMMARY

The cockle *Cerastoderma edule* plays a vital role both economically and ecologically along the coastal systems of the Northeast Atlantic, with increasing interest in aquaculture. Cockles are impacted by parasites that alter their population dynamics throughout their geographic range. One example is the trematode *Bucephalus minimus,* which uses cockles as first intermediate host. Trematodes are prevalent parasites in coastal systems, with a complex life cycle involving free-living and parasitic stages, infecting multiple hosts. Trematode infections can detrimentally affect host fitness, including growth, reproduction, and biochemical performance, potentially impacting ecosystem functioning. Nonetheless, the severity of these effects varies depending on the species and abundance of the parasite, as well as the stage of infection and interaction with the host's vital organs. *Bucephalus minimus* is one of the most deleterious parasites along the cockles' distributional range. It typically infects the gonad and digestive gland causing castration and energy depletion, leading to digestive tract autolysis and death. This study compares the gene expression profile of cockles chronically infected by *B. minimus* compared to non-infected cockles using RNA-seq analysis. Haemolymph from 10 infected and 10 non-infected cockles from Ramallosa Lagoon (Spain) was analysed, revealing significant differences in gene expression, with 870 up-regulated and 344 down-regulated genes in infected individuals. Cell-division and growth were the most impaired pathways. Understanding the genetic basis of bivalve response to parasite infection is crucial for conservation efforts. Furthermore, the comprehensive study of host-parasite dynamics can lead to the development of novel approaches to control disease outbreaks and mass mortalities.

Keywords: RNA-seq, *Bucephalus minimus*, Immune response, host-parasite interaction, differentially expressed genes

P49- DIFFERENTIAL GENE EXPRESSION AT DIFFERENT STAGES OF GONADAL DEVELOPMENT IN MALE AND FEMALE SENEGALESE SOLE (*Solea senegalensis*)

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SUMMARY

Solea senegalensis is one of the most promising and appreciated European aquaculture species due to its high commercial value and excellent meat quality. One of the principal cultivation challenges is the control of reproduction, including the identification and characterisation of genes involved in sex determination and gonadal development. A comparative analysis of gene expression by RNAseq between male and female gonads at different developmental stages (84, 98, and 126 days, juveniles, and mature adults) was carried out. Furthermore, a set of genes known to be involved in fish sex determination and differentiation was evaluated in these samples. RNAseq analyses indicated a temporal lag in sexual development between sexes. At 84-days and 96-days post fertilization (dpf), samples formed a single cluster according to their gene expression pattern with no differentiation between sexes. However, female samples at 126 dpf exhibited a similar expression pattern to juvenile males, clustering together. The 126 dpf male samples clustered with those at 84 and 96 dpf samples. Finally, the juvenile female samples clustered separately from all other clusters, while the male and female adult samples were found in a separate and simple cluster. This lag was also observed when specific genes involved in the sexual development were analysed. *gsdf*, *fshr*, and *ar* exhibited overexpression in males, specially at the juvenile stage. However, *aromatase*, *amh-r2*, and *vasa* exhibited overexpression in females, but with the greatest differences at 126 dpf.

Keywords: Senegal sole, RNAseq, sex determination, and gonadal development

P50- GENETIC DIVERSITY OF *Plesionika edwardsii*: A SHRIMP SPECIES WITH ECONOMIC INTEREST IN ALBORAN SEA REGION

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SUMMARY

Soldier shrimp (*Plesionika edwardsii*) is a marine crustacean with a worldwide distribution, being an important fishing resource in the occidental Mediterranean (Alboran Sea). In this enclave, different hydrographic regimes, and marine currents, could be causing the isolation of its populations impeding the flow between them. This could provide specific characteristics to the populations in this region, which could be exploited for commercial purposes. A total of 128 samples from three populations in the Alboran Sea (Mediterranean) and two populations in the Atlantic (Bay of Cadiz and Canary Islands) were analysed using 2b-RAD sequencing to assess genetic structure. We assembled a draft genome to serve as a reference for the alignment of filtered DNA reads in the search for SNPs. Our estimation of the size of the haploid genome was around 17 Gb. We achieved 95.2±0.2% read alignment with this reference genome using the Bowtie v1.3.1. Multimapped reads (number of reportable alignments > 1) were not used to build RAD-loci with Stacks v2.65, which detected 1,976,387 putative SNPs. After quality filtering steps, a total of 17,416 SNPs were retained and used to evaluate the genetic diversity and structure in the studied populations. The genetic diversity was similar between populations (He ~0.17). The data obtained showed no genetic differentiation among samples in the Alboran Sea, but a significant differentiation between the Alboran Sea and the two Atlantic populations. However, our analysis revealed that the greatest genetic differentiation occurs between the two Atlantic populations (Bay of Cadiz and Canary Islands; F_{ST} = 0.016).

Keywords: *Plesionika edwardsii*, Alboran Sea, SNP, genetic differentiation

P51- COMPARATIVE ANALYSIS OF GONADS FROM WILD AND F1 CULTIVATED ADULT MALES OF SENEGALESE SOLE BY scRNASeq

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SUMMARY

The main constraint in Senegalese sole (*Solea senegalensis*) farming is the low reproductive performance of farm-reared F1 males. This represents a significant obstacle to the growth of the industry, as it severely limits the implementation of selective breeding programmes. To address this problem and to elucidate the changes in the gonads of these infertile F1 males compared to wild fertile males, we have conducted a study comparing the gene expression of their gonads by scRNAseq (10X Chromium). Four samples for adult males, two from F1 cultivated and two from wild origin, were analysed. The sequencing data were processed using STAR software for alignment of the sequences. To ensure data quality, a threshold of 0.20 for the ratio of mitochondrial DNA, nCounts (reads) > 500, and nFeatures (UMIs) > 300 was set. A total of 80,161 cells were retained and logarithmic normalisation was applied. Subsequent analyses were conducted using the Seurat R package for clustering. The analyses identified specific cell subtypes in the gonad, which showed frequency variations between wild and farm-reared individuals. The main clusters were assigned to specific group cells by marker genes. The anti-Müllerian hormone gene characterised an expanded mature population in the wild-type as compared to the farm-reared F1 males. Further analysis was conducted on the expanded clusters, which revealed significant aberrant biological pathways involved in the lack of maturation in the gonads of the farm-reared males.

Keywords: Senegalese sole, reproduction, farm-reared F1 males, scRNAseq

P52- MORPHOMETRICS IN DIFFERENT GENOTYPIC CLASSES OF *DICENTRARCHUS LABRAX* TWO DEVELOPMENTAL STAGES (LINNAEUS, 1758)

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SUMMARY

The European seabass is a widely farmed fish species in Europe and particularly in the Mediterranean Sea. Analyses of genomic data from wild and farmed Mediterranean populations identified candidate genes (including *six6* and *bcl6ab*) as potential targets of genetic breeding programs. The aim of this study was to investigate the correlation of the SNPs of the *six6* and *bcl6ab* genes with the growth of farmed individuals. Two developmental stages (larvae-34 days post-hatching, dph, and juveniles-71 dph) were analysed. Classical morphometrics were employed to assess the effect of the studied SNPs (two SNPs in *six6* and one SNP in *bcl6ab*) on the morphology. The statistical analysis of 20 characters was performed for four combinations of *six6* and three *bcl6ab* genotypic classes in the larval stage and 26 morphometric characters for five combinations of *six6* and two *bcl6ab* genotypic classes in the juvenile stage by applying multivariate and univariate analyses in the IBM SPSS program. Differences in shape and size, without and with the effect of allometry, were investigated and significant effects of SNPs on them were found. In particular, the effect of *six6* increased in the juvenile stage, while the reverse was observed for *bcl6ab*. *Six6* mainly affects the length and morphology of the tail, while bcl6ab affects the thickness and morphology of the fins. Similarly, the individuals with *six6* homozygous genotypes presented greater body length and the *bcl6ab* homozygous genotypes presented increased thickness. These findings thus support previous genome scans between wild and farmed populations at the phenotypic level.

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Keywords: European seabass, *six6*, *bcl6ab*, morphometrics, aquaculture

Presenting Author Index

Chen, Y.L.	Comparison of tendon tissue differentiation between zebrafish and medaka by single-cell sequencing	Huazhong Agricultural University	Poster	79
Choresca, C. H. Jr.	Molecular identification of bacterial pathogens associated with diseases in aquaculture farms in the Philippines	NFRDI	Poster	93
Clark, T.	Conserved and divergent arms of the antiviral response in the duplicated genomes of salmonid fishes	University of Aberdeen	Oral	18
Cnaani, A.	Transcriptomic adaptation to salinity in Mozambique tilapia takes shorter time than in Nile tilapia	Volcani Institute	Oral	55
Correia, S.	Gene expression profile of the cockle *Cerastoderma edule* in response to trematode infection	CIIMAR	Poster	107
Costas-Imbernon, D.	Understanding the genetic basis of pigmentation anomalies in flatfish	IIM-CSIC	Poster	94
David, L.	Due to their improved immunity, disease resistant common carp fish are also less infective	The Hebrew University of Jerusalem	Poster	86
De la Herrán, R.	Differential gene expression at different stages of gonadal development in male and female Senegalese sole (*Solea senegalensis*)	University of Granada	Poster	108
Duperret, L.	Low metabolism activity and efficient antiviral response enable to counteract the pacific oyster mortality syndrome in non-permissive conditions induced by the temperature	Ifremer	Oral	29
Estêvão, J.	Signatures of selection for tolerance and resistance to *Perkinsus olseni* in grooved carpet shell clam (*Ruditapes decussatus*) using population genomics	University of Santiago de Compostela	Oral	30
Fernández, I.	A heatwave event after hatching alters DNA methylation profile in rainbow trout (*Oncorhynchus mykiss*)	IEO-CSIC	Poster	85
Fernández-Boo, S.	GRINNAQUA: green innovation strategies for animal health management: towards sustainable aquaculture	CIIMAR	Poster	96
Florea, A.	Single nuclei RNA-sequencing of WSSV-infected whiteleg shrimp (*Litopenaeus vannamei*) reveals novel genes involved in white spot infection	The Roslin Institute	Oral	28
Fraslin, C.	Genetic analysis of growth traits in fugu, *Takifugu rubripes*	The Roslin Institute	Poster	81

Guerrero-Peña, L.	Integration of multi-omics data to uncover key genes and metabolic pathways involved in flatfish metamorphosis	IIM-CSIC	Oral	45
Gulliksen, S.	Influence of 3'UTR variants on germ cell-specific gene expression in Atlantic salmon	Mowi Genetics	Poster	67
Henkel, C.	SALMOCODE: A single-cell atlas of Atlantic salmon organogenesis	Norwegian University of Life Sciences	Oral	42
Kokou, F.	Early life feeding effects on Nile tilapia gut microbiome and transcriptome	Wageningen University	Oral	58
Konstantinidis, I.	Single-cell transcriptome and epigenetic profiling of the pituitary gland provide insights into teleost puberty	Nord University	Poster	101
Krasnov, A.	Immunoglobulin sequencing provides new insight in B cells biology of salmonid fish	Nofima	Oral	17
Kriaridou, C.	Impact of the incorporation of functional annotation into genomic prediction in a turbot population challenged with *Philasterides dicentrarchi*	The Roslin Institute	Oral	23
Kurta, K.	Genomic regions associated with variable sperm motility across the reproductive season in Artic charr	Swedish University of Agricultural Sciences	Oral	38
Kurta, K.	First insights into genetic differentiation among sympatric morphs of arctic charr based on whole-genome sequencing	Swedish University of Agricultural Sciences	Oral	50
Lazado, C.C.	The molecular and cellular repertoires of a teleost tongue – insights into the interplay of gustation and immunity	Nofima	Oral	14
Liu, Z.J.	Epigenetic regulation of genetic sex determination of channel catfish explains sex ratio variations with high temperature	Tennessee Tech University	Oral	34
Luo, L.F.	Genetic analysis and development of sex-specific markers in populations of *Megalobrama amblycephala* based on whole-genome re-sequencing	College of Fisheries Huazhong Agricultural University	Poster	88
Malandrakis, E.E.	The effect of dietary *Rhodotorula mucilaginosa* on liver antioxidant gene expression of *Sparus aurata*	Agricultural University of Athens	Poster	95
Malysheva, O.	Genetic identification of sturgeon populations from Ukraine using DNA markers	The Leibniz Institute for Zoo and Wildlife Research	Poster	97

Manousi, D.	Dissecting the genetic basis underlying response against salmonid alphavirus in Atlantic salmon	Norwegian University of Life Sciences	Poster	71
Marcoli, R.	Correlation between epigenetic and transcriptomic changes in golden barramundi (*Lates calcarifer*): a multiomic approach	James Cook University	Oral	47
Marcos-Hadad, E.	A new sex determining gene identified in striped bass	The Hebrew University of Jerusalem	Poster	87
Martínez, P.	High-throughput screening of the first chromosome-level genome assembly of *Merluccius merluccius* suggests a sex determining gene underlying a XX/XY system	University of Santiago de Compostela	Oral	35
Mehta, T. K.	Chromatin accessibility in gill tissue identifies candidate genes and loci associated with aquaculture relevant traits in tilapia	Earlham Institute	Oral	21
Moulistanos, A.	Unveiling genomic signatures for domestication in European seabass (*Dicentrarchus labrax L.*)	Aristotle University of Thessaloniki	Oral	51
Moulistanos, A.	Gene expression profiling of six6 and bcl6a in larva and juvenile stages in European seabass (*Dicentrarchus labrax* L.)	Aristotle University of Thessaloniki	Poster	89
Moulistanos, A.	Morphometrics in different genotypic classes of *Dicentrarchus labrax* two developmental stages (Linnaeus, 1758)	Aristotle University of Thessaloniki	Poster	111
Mukiibi, R.	Functional genomic architecture of viral nervous necrosis disease resistance in farmed European seabass	The Roslin Institute	Oral	24
Naya-Catala, F.	Using long-read sequencing with oxford nanopore to enable genomic and epigenomic profiling of livestock fish	IATS-CSIC	Poster	90
Naya-Catala, F.	The expansion of sirtuin gene family in gilthead sea bream (*Sparus aurata*). Phylogenetic, syntenic and functional insights across the vertebrate/fish lineage	IATS-CSIC	Poster	91
Oikonomou, S.	GWAS and accuracy of prediction for growth in meagre *Argyrosomus regius*	HCMR	Oral	59

Oikonomou, S.	Combined quantitative and population genetics methods provide insight into the genetic landscape of fillet fat in European sea bass *Dicentrarchus labrax*	HCMR	Poster	77
Pampin, M.	Genetic resilience in the common cockle (*Cerastoderma edule*): contrasting natural selection and selective breeding for marteiliosis resistance	University of Santiago de Compostela	Poster	83
Panteli, N.	Assessing the impact of insect meal on the intestinal bacterial microbiota of the Baltic shrimp *Palaemon adspersus*	Aristotle University of Thessaloniki	Poster	103
Papadaki, M.	Differential expression of micro RNAs (miRNAs) in gonads of gilthead seabream Sparus aurata	HCMR	Poster	92
Pappas, F.	Deciphering the sperm methylation landscape of Swedish Arctic charr with regard to male-fertility traits	Swedish University of Agricultural Sciences	Oral	39
Perera, E.	Genome survey for processes susceptible to manipulation through DNA methylation remodelling in gilthead sea bream	ICMAN-CSIC	Oral	62
Piazzon, M.C.	Deciphering the IGH locus of gilthead seabream	IATS-CSIC	Poster	64
Piferrer, F.	DNA methylation during early development in diploid and triploid European seabass	ICM-CSIC	Oral	44
Rebordinos., L.	Molecular characterization of breeders of *Solea sengalensis*	University of Cádiz	Poster	98
Regan, T.	Applying a chromosome-scale assembly to explore population structure, metagenomics and evolution in the blue mussel (*Mytilus edulis*)	The Roslin Institute	Poster	84
Rey-Campos, M.	Transcriptomic approach to characterize gene expression in mussel (*Mytilus galloprovincialis*) gills and hemocytes from the hemolymph or the intervalvar liquid	IIM-CSIC	Oral	25
Ribas, L.	Unravelling sex-dependent epigenetics mechanisms in response to environmental cues	ICM-CSIC	Oral	40
Riesco, M.F.	A single heatwave event is able to disrupt spermatogenesis in rainbow trout (*Oncorhynchus mykiss*) breeders	University of León	Poster	99

Robinson, N.A.	Use of transcriptomics and gene editing to explore genetic mechanisms giving species-specific host-resistance to sea lice	Nofima	Oral	16
Robles, F.	Genetic diversity of *Plesionika edwardsii*: a shrimp species with economic interest in the Alboran sea region	University of Granada	Poster	109
Rodríguez-Vázquez, R.	A high-quality genome assembly of the Mediterranean mussel (*Mytilus galloprovincialis*) provides insights into mussel sex determination	University of Santiago de Compostela	Poster	100
Romero, A	Transcriptomic analysis of turbot (*Scophthalmus maximus*) treated with zymosan a reveals that lncRNAs and inflammation-related genes mediate the protection conferred by β-glucans against *Aeromonas salmonicida*	IIM-CSIC	Poster	69
Ruiz-Rejón, C.	Comparative analysis of gonads from wild and F1 cultivated adult males of Senegalese sole by scRNASeq	University of Granada	Poster	110
Saco, A.	The genomic basis of the effective immune response of mussels	IIM-CSIC	Oral	26
Saco, A.	Mussel antiviral transcriptome response and elimination of viral haemorrhagic septicaemia virus (VHSV)	IIM-CSIC	Poster	68
Salisbury, S.J.	Genomic basis of the variation in sea lice susceptibility among Atlantic salmon and coho salmon revealed by single-nuclei RNA sequencing	The Roslin Institute	Oral	15
Samaras, A.	Molecular and physiological responses to chronic stress in European sea bass, *Dicentrarchus labrax*	University of Crete	Poster	72
Samaras, A.	Early life programming and molecular regulation of the stress response in greater amberjack (*Seriola dumerili)* larvae	University of Crete	Poster	80
Sambade I. M.	Validation of a genomic region associated with bonamiosis status in flat oyster populations at individual level	University of Santiago de Compostela	Oral	27
Shankregowda, A.	Microbiome responses to aquaculture-related husbandry practices	Swansea University	Oral	19

Skjærven, K.H.	Broodstock management influences the metabolic status, gene expression and epigenetic gene regulation in progeny	University of Bergen	Oral	49
Tadmor-Levi, R.	Utilizing pool-seq to identify the gene controlling black patches pigmentation in ornamental carp (koi)	The Hebrew University of Jerusalem	Oral	61
Torres, D.	Characterisation of olfactory transcriptome in Senegalese sole (*Solea senegalensis*) at the single-cell level: functional implications in reproduction	University of Santiago de Compostela	Oral	36
Toxqui-Rodríguez, S.	Host-microbiota cross-talk upon gut parasite infection in gilthead seabream	IATS-CSIC	Oral	20
Tsipourlianos, A.	Evolutionary dynamics of paralogous genes in oxidative phosphorylation and ontogenetic regulation in gilthead seabream (*Sparus aurata*)	University of Thessaly	Poster	82
Wan, S.	Study on the developmental characteristics and molecular regulatory mechanisms of different types of intermuscular bone in *Coilia nasus*	Huazhong Agricultural University	Poster	104
Xu, P.	Applications of genomic selection for complex economic traits in large yellow croaker	Xiamen University	Oral	33
Yu, Y.	Identification of SNPs and candidate genes associated with growth using GWAS and transcriptome analysis in *Coilia nasus*	Huazhong Agriculture University	Oral	60
Żarski, D.	Mitochondrial function is at the frontline of domestication in Eurasian perch (*Perca fluviatilis*)	Polish Academy of Sciences	Oral	52
Žuvić, L.	Different sampling approaches for DNA metabarcoding analysis of the stomach contents of the Atlantic bluefin tuna (*Thunnus thynnus* Linnaeus, 1758)	IZOR	Poster	66

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Ευχαριστούμε!

Thank you!

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