6TH INTERNATIONAL SYMPOSIUM ON GENOMICS IN AQUACULTURE

DATE
5-7 MAYO
2021

GIA 2020

BOOK OF ABSTRACTS

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6TH INTERNATIONAL SYMPOSIUM ON GENOMICS IN AQUACULTURE

DATE 5-7 MAYO 2021 IN GRANADA

GIA 2020

DAY 1

	9:45	**GIA WELCOME**		
WEDNESDAY		**Session 1: Genomic developments for aquaculture research**	**Chair: Elisavet Kaitetzidou**	**Sponsored by Illumina**
10:00-12:15	10:00	Genome, transcriptome and biotechnological potential of *Mytilus galloprovincialis*	Antonio Figueras	IIM-CSIC (Spain)
	10:15	A chromosome-level reference genome assembly for the pacific oyster (*Crassostrea gigas*)	Carolina Peñaloza	University of Edinburgh (UK)
	10:30	Giant river prawn *Macrobrachium rosenbergii* enters the genomic era, revolutionizing freshwater prawn industry	Amir Sagi	Ben-Gurion University of Negev (Israel)
	10:45	Advancing molluscan cell culture for aquaculture and genomics applications	Robert Potts	University of Edinburgh (UK)
		Coffe break (10 min)		
	11:10	Highly efficient genome editing in salmonid cell lines using cas ribonucleoprotein	Yehwa Jin	University of Edinburgh (UK)
	11:25	Using nuclei to explore the genomic regulation and cell specific basis of gene expression in Atlantic salmon	Rose Daniels	University of Edinburgh (UK)
	11:40	Genomic and sexual determination studies in sole (Solea senegalensis) through map integration	Rafael Navajas-Perez	University of Granada (Spain)
	11:50	Circular rna host gene prediction in nile tilapia using the novel CircParser pipeline	Artem Nedoluzhko	Nord University (Norway)
	12:00	Applications of Illumina Technologies to Aquaculture	Andre Eggen	Illumina
		Session 2: Host-pathogen interactions	**Chair: Aurora Campo**	
13:30-15:40	13:30	Advantages and disadvantages of zebrafish as a model for aquacultured species	Beatriz Novoa	IIM-CSIC (Spain)
	13:45	Characterisation of genetic resistance to infectious salmon anaemia virus in Atlantic salmon	Ophélie Gervais	University of Edinburgh (UK)
	14:00	Transcriptomic response of mussel gills against a bath infection with *Vibrio splendidus*	Amaro saco	IIM-CSIC (Spain)
	14:15	Identification of functional genes related to host resistance to *Piscirickettsia salmonis* in atlantic salmon (*Salmo salar*) using RNA-seq	Carolina Moraleda	University of Edinburgh (UK)
		Coffe break (10 min)		
	14:40	RNA-Seq analysis of European sea bass (*Dicentrarchus labrax* L) infected with nodavirus reveals powerful modulation of the stress response	Raquel Lama	IIM-CSIC (SPAIN)
	14:55	Phagocytic activity and marker gene expression of the adherent cells from intestine and head kidney of Atlantic salmon	Youngjin Park	Nord University (Norway)
	15:10	Expansion of gene families throughout bivalve molluscs evolution	Tim Regan	University of Edinburgh (UK)
	15:25	Evaluation of immune-response biomarkers in gilthead seabream fed with novel feed formulations	Ana Fernandes	Nord University (Norway)

DAY 2

THURSDAY		**Session 3: Selective breeding and evolutionary genetics**	**Chair: Ioannis Konstantinidis**	
10:00-12:10	10:00	Genomic selection for white spot syndrome virus resistance in whiteleg shrimp (*Litopenaeus vannamei*) boosts survival under an experimental disease challenge test	Nick Robinson	Nofima (Norway)
	10:15	Population genomics and adaptive evolution of Chinese seabass *Lateolabrax maculatus*	Peng Xu	Xiamen University (China)
	10:30	Biotic and abiotic factors shaping the genome of cockles (*Cerastoderma edule*) in the Atlantic area	Manuel Vera	University of Santiago de Compostela (Spain)
	10:45	Balancing selection at the ATP binding site of heat shock cognate 70 (HSC70) contributes to increased thermotolerance in *Artemia franciscana*	Peter Bossier	Ghent University (Belgium)
		Coffe break (10 min)		
	11:10	Old and recent inbreeding effects on female size and reproduction traits in a rainbow trout selected line	Katy Paul	INRAE (France)
	11:25	Benefits of genomic evaluation in aquaculture breeding programs when tank effects are present	Jesús Fernández	INIA (Spain)
	11:40	Genetic selection for growth drives differences in intestinal microbiota composition and parasite disease resistance in gilthead sea bream	Carla Piazzon	IATS-CSIC (Spain)
	11:55	Does crossbreeding influence the intestinal bacterial composition of Nile tilapia?	Yousri Abdelhafiz	Nord University (Norway)
		Session 4: Sex differentiation and reproduction	**Chair: Yehwa Jin**	
13:30-15:40	13:30	Analysis of repetitive sequences in the *Solea senegalensis* genome and their role in the evolution of a proto sex-chromosome	Laureana Rebordinos	University of Cadiz (Spain)
	13:45	Fine mapping of QTL associated with spontaneous masculinisation in XX-female rainbow trout	Clemence Fraslin	INRAE (France)
	14:00	Single-cell RNA-Seq identifies hormone-producing cell types in the teleost pituitary	Khadeeja Siddique	NMBU (Norway)
	14:15	Ectopic expression of lipid homeostasis genes in the teleost pituitary gland during sexual maturation	Christian Henkel	NMBU (Norway)
		Coffe break (10 min)		
	14:40	Persistent gonadal epigenetic changes due to elevated temperature during early development in zebrafish (*Danio rerio*)	Francesc Piferrer	ICM-CSIC (Spain)
	14:55	Multigenerational epigenetic inheritance of masculinizing temperatures in the European sea bass	Núria Sánchez-Baizán	ICM-CSIC (Spain)
	15:10	Non-coding RNAs are involved in the regulation of teleost gonad maturation	Maria Papadaki	HCMR (Greece)
	15:25	Identification of conserved sex-specific micrornas in teleost species and their functionality in zebrafish gonads	Tosca van Gelderen	ICM-CSIC (SPAIN)

DAY 3

FRIDAY		**Session 5: Growth, development and stress**	**Chair: Tim Regan**	**Sponsored by Sparos**
10:00-12:45	10:00	Exploring early development of a model and an economically important species through miRNA's expression	Elisavet Kaitetzidou	HCMR (Greece)
	10:15	Targeting the mild-hypoxia driving force for metabolic and muscle transcriptional reprogramming of gilthead sea bream (*Sparus aurata*) juveniles	Fernando Naya-Català	IATS-CSIC (Spain)
	10:30	Seasonal broodstock management influences the nutritional status, gene expression and epigenetic gene regulation in Atlantic salmon progeny	Kaja Skjærven	Institute of Marine Research (Norway)
	10:45	Differences in liver DNA hydroxymethylation reflect the growth phenotype	Ioannis Konstantinidis	Nord University (Norway)
	11:00	Different transcriptomic strategies to cope with salinity challenge in Nile and Mozambique tilapias respond to sympatric speciation	Aurora Campo	Agricultural Research Organization (Israel)
		Coffe break (10 min)		
	11:25	Exogenously generated reactive oxygen species alter the transcriptomic landscape of Atlantic salmon olfactory mucosa	Carlos Lazado	Nofima (Norway)
	11:40	The mitochondrial 5-methylcytosine profile in Nile tilapia (*Oreochromis niloticus*)	Artem Nedoluzhko	Nord University (Norway)
	11:55	Applied hologenomics: leveraging microbiota services through holo-omic analyses in farmed fish	Morten Limborg	University of Copenhagen (Denmark)
	12:10	Intestinal transcriptome of zebrafish fed soybean and yeast β-glucan	Saima Rehman	Nord University (Norway)
	12:20	Dietary glucans can restore normal lipid metabolism in zebrafish	Adnan Gora	Nord University (Norway)
	12:30	SPAROS: application of molecular biomarkers to fish nutrition	Tomé Silva	Sparos
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GENOME, TRANSCRIPTOME AND BIOTECHNOLOGICAL POTENTIAL OF *Mytilus galloprovincialis*

Figueras, A., Romero, A., Rey-Campos, M., Saco, A., Moreira, R. and Novoa, B.

Institute of Marine Research (IIM), National Research Council (CSIC), Vigo, Spain.

SUMMARY

The Mediterranean mussel (*Mytilus galloprovincialis*) is a marine species cultured all over the world. As all invertebrates, mussels lack an adaptive immune system but they respondto pathogens, injuries or environmental stress in a very efficient manner. In fact, according to their filtering-feeding nature, mussels are constantly exposed to pathogens, but scarce mortality has been registered in natural environments. The sequencing of the mussel genome has revealed a very complex organization with high heterozygosity, abundance of repetitive sequences and extreme intraspecific sequence diversity among individuals, mainly in immune related genes. Among those genes, antimicrobial peptides are the most expressed gene families in mussels, highly polymorphic and with antimicrobial effectagainst mollusks pathogens, but also against pathogens of lower vertebrates and humans.We demonstrated that myticin C also presents properties related to the biotechnological application of in the wound healing of vertebrates, including humans. The combination of a complex genome with the adaptation of mussel immune system to a changing environment could explain mussel genomic variability and its powerful immune system.

A CHROMOSOME-LEVEL REFERENCE GENOME ASSEMBLY FOR THE PACIFIC OYSTER (*Crassostrea gigas*)

Peñaloza, C[1]., Gutierrez, A[1]. Eory, L[1]., Archibald, A[1]., Bean, T[1]. and Houston, R.D[1].

1. *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH25 9RG, United Kingdom*

SUMMARY

Pacific oysters (*Crassostrea gigas*) are one of the world's most important aquaculture species, contributing to 98% of global oyster production. Selective breeding for genetic improvement of oysters is at a formative stage and genomic tools are beginning to be applied. High-quality annotated reference genomes are key genomic resources that enable genetics and breeding research, as they provide the foundation for understanding the molecular basis of important production traits. In 2012, a reference genome was developed for the Pacific oyster. Although this reference has been used for a multitude of studies, it is highly fragmented and contains numerous misassembled scaffolds. Therefore, an improved reference genome assembly is highly desirable to support genetics and genomics research in oysters.

The aim of this study was to develop a high-quality Pacific oyster genome assembly by making use of long-read sequencing and scaffolding technologies. A single female oyster was sequenced at a high coverage (~80x) using Pacific Biosciences technology together with Illumina sequencing for error correction. After processing the sequence data using the Canu algorithm, an initial assembly that was considerably larger than expected was obtained, likely due to the high levels of genome heterozygosity observed in oysters. Highly divergent haplot33ypes were detected in this preliminary assembly and reassigned using a combination of the 'purge haplotigs' pipeline and an 'all-versus-all' contig mapping approach. Following scaffolding using Hi-C sequence reads and the integration of the scaffolds with a high-density linkage map (~20K SNPs), a chromosome-level assembly with a scaffold N50 of 58.4Mb was obtained. This new reference genome will be a valuable tool for use of genomic information in breeding of Pacific oysters, and will also help provide insight into the unusual genome biology of this species.

GIANT RIVER PRAWN *Macrobrachium rosenbergii* ENTERSTHE GENOMIC ERA, REVOLUTIONIZING FRESHWATER PRAWN INDUSTRY

Dotan, S[1]. Rosen, O[1]. Baruch, K[5]. Levy, T[2]. Manor, R[2]. Aflalo, E.D[3]. Shechter, A[1]. Sagi A[2,4].

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2. *Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*
3. *Department of Life Sciences, Achva Academic College, Israel*
4. *The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*
5. *NRGene Technologies 2009 Ltd., Golda Meir St., Ness-Ziona 7403649 Israel*

SUMMARY

The first proprietary technology which supplies broodstock to produce all-female giant prawn *Macrobrachium rosenbergii* populations was developed and commercialized by Enzootic. This novel technology, based on a single androgenic gland cell transplantation, have demonstrated the advantages of all-female prawn aquaculture in all parameters evaluated in a large field study such as growth rate, harvest size, FCR and reduced reproductive activity. It is performed without undesired interventions such as genetic modifications, exogenous hormones or hazardous chemicals. In the absence of dominant males, the uniformity of the product and the presumable reduction of territoriality and aggressiveness are offering unique benefits in further intensified prawn culture and potential use of RAS systems.

Proprietary genomic sex markers together with the first assembly of a high-quality unphased and an independently assembled phased genome exhibiting distinguishable paternal and maternal sequences, enabled identification and validation of the W and Z chromosomes in prawns. The assembly quality of both the phased and unphased genome is superior to any crustacean genome assembled to date. The genome size of the phased and unphased genome is 6.6Mb and 3.5Mb, respectively, their N50 is 19Mb and 1.7Mb, respectively and their BUSCO score was 87.8% and 92.7%, respectively.

For the purpose of future selective breeding, we have characterized the genetic variance of two breeding lines, one relatively narrow (WW - maternal) and another heterogenic (WZ/ZZ- paternal) and validated the universality of our sex markers. An effort to further characterize novel SNP's on autosomal chromosomes with disequilibrium to selective traits is ongoing and a genomic chip is being assembled. This sets the ground for a genomic based selective breeding of the all-female population for enhanced performance lines, adapted to high intensity growing conditions in prawn aquaculture.

ADVANCING MOLLUSCAN CELL CULTURE FOR AQUACULTURE AND GENOMICS APPLICATIONS

Robert W.A. Potts[1,2], Alejandro P. Gutierrez[1], Yennifer Cortés-Araya[1], Ross D. Houston[1],Tim P. Bean[1]

1. *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom*
2. *Centre for Environment Fisheries and Aquaculture Science (Cefas) Weymouth Laboratory, United Kingdom*

SUMMARY

Pacific oysters are one of the world's most important aquaculture species, and are considered as model bivalve mollusc. However, infectious disease outbreaks are a major threat to global production, causing periodic mass mortalities. As with other marine invertebrate species, cell culture systems to support research into disease, genetics, and physiology are seriously limited (1). Primary cell and tissue cultures are typically used but remain poorly characterized,which can cause issues with experimental consistency and reproducibility. improvements to methods of repeatable isolation, culture, and characterization of oyster cells and tissues are required to help address these issues.

In the current study, systematic improvements have been developed to facilitate the culture of primary cells from adult Pacific oysters, including several different tissues. Cultures analysed by light microscopy, qPCR, and live cell imaging demonstrated maintenance of live, metabolically active cells for several weeks post-explant. Interestingly, whole hearts dissected from adult oysters were found to continue contracting rhythmically up to 8 weeks after being transferred to a tissue culture system. Mantle tissue explants were also actively moving in the culture system.

These improved primary cell cultures and the new whole tissue culture systems have many potential applications for aquaculture and genomics research. This may include culture and characterisation of pathogens, leading to more controlled experimental conditions for disease challenges, as has been applied to study disease in finfish aquaculture (2). Specifically, the dynamics between Pacific oysters and ostreid herpes virus (OsHV; a frequent cause of mass mortality events) are poorly understood and could be elucidated through the use of culture-based experiments.

1. Yoshino TP, Bickham U, Bayne CJ. Molluscan cells in culture: primary cell cultures and cell lines. Can J Zool. 2013;91(6):391-404.
2. Houston RD, Macqueen DJ. Atlantic salmon (Salmo salar L.) genetics in the 21st century: taking leaps forward in aquaculture and biological understanding. 2019;50(1):3-14

HIGHLY EFFICIENT GENOME EDITING IN SALMONID CELLLINES USING CAS RIBONUCLEOPROTEIN

Jin, Y.H., Gratacap, R.L., Mantsopoulou, M. and Houston, R.D.

The Roslin Institute, The University of Edinburgh, United Kingdom

SUMMARY

Salmonid fish such as Atlantic salmon and rainbow trout are important aquaculture species which international trade values account for 18 percent of the total traded value of fish product in 2017 (FAO, 2019). Selective breeding programmes have enabled major gains in production traits to date, and genome editing has potential for further step improvements (Gratacap et al., 2019a). Disease resistance is one of important production traits and understanding of genetic basis of variation in resistance to pathogens is crucial. Identification of candidate genes or mutations identified from genome-wide association studies can be enabled by CRISPR/Cas systems in fish cell lines. CRISPR/Cas systems can also be used for high throughput genome wide screening to identify functional genes which explain the disease susceptibility in easy-to-transduce cell lines such as CHSE214-EC (Dehler et al., 2016;Gratacap et al., 2019b). However, efficient methods for genome editing of cell lines derived from Atlantic salmon and rainbow trout has not yet been reported, and the aim of the current study was to address this gap. Four salmonid cell lines were studied (Atlantic salmon head kidney, SHK-1; Atlantic salmon kidney, ASK; rainbow trout gonad, RTG-2; Chinook salmon embryo, CHSE214). Firstly, optimisation of concentration of Cas9 ribonucleoprotein (RNP), electroporation settings and incubation time was performed using SHK-1. The optimised Cas9 RNP electroporation settings were then tested in ASK, RTG-2 and CHSE214 with very minor adjustments. Gene knockout (KO) by genome editing was also tested using Cas12a RNP, noting that Cas12a hasa different PAM site of 5'TTTV to Cas9 of 5'NGG, expanding the potential target sites in the species' genomes. The efficiency of editing and the nature of the edits was measured by Sanger sequencing of samples from each of the edited cell lines followed by ICE (Synthego Inc.) or TIDE analysis (Brinkman et al. 2014). The optimised Cas9 RNP electroporation resulted in 91 – 97 % occurrence of indel mutations in SHK-1, ASK and RTG-2 and 77 % indel in CHSE214 using an identical guide RNA targeting exon 6 of the *slc45a2* gene. Cas12a RNP was also successful in producing targeted edits with a crRNA targeting the same exon (65 % indel) but was less efficient than Cas9RNP in the SHK-1 and RTG-2.
The results highlight a simple, quick, yet powerful genome editing method for salmonid cell lines by employing Cas RNP complexes and electroporation. This method will assist with functional analysis of candidate targets to understand genetic disease resistance in salmonid species.

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Brinkman, E.K., Chen, T., Amendola, M., van Steensel, B., 2014. Easy quantitative assessmentof genome editing by sequence trace decomposition. Nucleic Acids Res. 42, e168

USING NUCLEI TO EXPLORE THE GENOMIC REGULATION AND CELL SPECIFIC BASIS OF GENE EXPRESSION IN ATLANTIC SALMON

Ruiz-Daniels, R., Taylor, R.S., and Macqueen, D.J.

The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK.

SUMMARY

Advanced genomic technologies are becoming increasingly available to interrogate the transcriptional regulation and cell-specific basis of gene expression in aquaculture species. During this talk, I will outline the recent successful uptake of ATAC-seq to assess chromatin genome-wide accessibility, and single nuclei RNA-Seq (snRNA-Seq) for single cell transcriptomics, in Atlantic salmon. These methods have been developed by our lab using nuclei from frozen or cryopreserved tissues. In addition to covering the key lab steps involved, I will outline a snRNA-Seq atlas of the Atlantic salmon liver, which has provided insights into the dynamics of different cell populations challenged by bacterial infection.

SOLGEN: GENOMIC AND SEXUAL DETERMINATION STUDIES IN SOLE (*Solea senegalensis*) THROUGH MAP INTEGRATION

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2. Departamento de Zoologia, Genética y Antropología Física. Facultad deVeterinaria. Universidad de Santiago de Compostela. Spain
3. IRTA, Dpt. De Producción Animal. Sant Carles de la Ràpita. Tarragona,Spain

SUMMARY

The Senegalese sole (*Solea senegalensis*, Kaup 1858) is a flatfish species distributed from Atlantic African and European coasts to western Mediterranean coasts and represents a very promising species for marine aquaculture. Its production increases every year mostly due to the rapid larval development and its high growth rate.

The construction of its first haploid genetic map (1), the identification of a pair of proto-sex chromosomes in its genome and the characterization of the dmrt1 gene (2, 3) are important landmarks in the generation of genomic information for this organism.

On these grounds, we are generating NGS for marker enrichment and construction of a high-resolution diploid genetic map. Nearly 30,000 SNPs markers were generated froma RAD library constructed from three reference families with offspring of 90 individuals each. The integration of these data will allow to better the genome featuresof Senegalese sole, (including sex-determination mechanism and the completeidentification of sex chromosomes), and to perform synteny analyses with other flatfish and model species genomes.

References

(1) Molina-Luzón et al., (2015). First Haploid Genetic Map Based on Microsatellite Markers in Senegalese Sole (, Kaup 1858). Marine Biotechnology, 17(1):8-22.
(2) Cross et al., (2020). The genomic structure of the highly-conserved dmrt1 gene in *Solea senegalensis* (Kaup, 1868) shows an unexpected intragenic duplication. PLoS One, 15(11): e0241518.
(3) Rodríguez et al., (2019). Evolution of the Proto Sex-Chromosome in *Soleasenegalensis*. Int J Mol Sci., 20(20):5111.

CIRCULAR RNA HOST GENE PREDICTION IN NILE TILAPIA USING THE NOVEL CIRCPARSER PIPELINE

Nedoluzhko, A.V.[1], Sharko, F.S.[2], Rbbani, Md.G.[1], Teslyuk, A.B.[2], Konstantinidis, I.[1], and Fernandes, J.M.O.[1]

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2. National Research Centre "Kurchatov Institute", Moscow, 123182, Russia

SUMMARY

De novo genome sequencing has become a routine procedure due to a decrease in sequencing costs, diversification of high-throughput sequencing platforms and improvement of bioinformatic tools. However, the quality of non-model species genome assemblies and, as a result, their annotations are often of unsatisfactory quality.

Circular RNAs (circRNAs) are long noncoding RNAs which play a significant role in various biological processes, including embryonic development and stress responses. A number of circRNA *de novo* and host gene prediction tools are available to date, but their ability to accurately predict circRNA host genes is limited in the case of low-quality genome assemblies or annotations.

Here we describe CircParser (https://github.com/SharkoTools/CircParser), a novel, easy to use Unix/Linux pipeline for circular RNA host gene prediction using the blastn program and the freely available bedtools software (Figure 1). CircParser is most useful for circRNA host gene prediction analysis in whole transcriptomic datasets of low-quality assemblies as well as poorly annotated genomes.

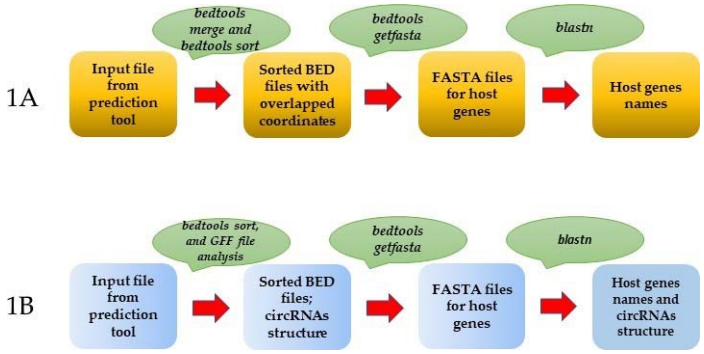

Figure 1. An overview of the CircParser pipeline.

We demonstrate the prediction capacity of CircParser on a recently published transcriptomic dataset from fast muscle of wild or domesticated female Nile tilapia (*Oreochromis niloticus*), using the five most popular circRNAs *in silico* prediction tools – CIRI, CIRI2, CircExplorer2, find_circ, and circFinder.

Acknowledgements: This study has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme [grant agreement no 683210] and from the Research Council of Norway under the Toppforsk programme [grant agreement no 250548/F20]. Fedor Sharko was supported by the RFBR (Russian Foundation for Basic Research) Grant no 19-04-00033.

SPONSOR TALK

APPLICATIONS OF ILLUMINA TECHNOLOGIES TO AQUACULTURE

Eggen, A.[1]

1. Illumina Inc., Hayward, California, United States of America.

ADVANTAGES AND DISADVANTAGES OF ZEBRAFISH AS AMODEL FOR AQUACULTURED SPECIES

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SUMMARY

Zebrafish (*Danio rerio*), largely used as a model for studying developmental processes, has also emerged as a valuable system for modeling human disease and also as a model for other aquacultured fish. Zebrafish possesses a complex immune system comparable to those of mammalian models. However, whole-genome duplication event and subfunction specialization of gene duplicates results in a more intricate relationship among the components implicated in the immune response and as a consequence in the response against diseases. In our work, we show how the real-time imaging and the use of the whole animal are excellent tools to visualize the in vivo interaction of a pathogen with the immune system. As a model, we investigated if the genomic responses of adult zebrafish induced by immune stimulation are conserved throughout evolution. Therefore,we confirm that zebrafish is an ideal model to study the basic mechanisms of immunity and the genomic responses of fish against diseases.

CHARACTERISATION OF GENETIC RESISTANCE TO INFECTIOUS SALMON ANAEMIA VIRUS IN ATLANTIC SALMON

Gervais O.[1], Barria A.[1], Papadopoulou A.[1], Gratacap R.[1], Moghadam H.[2], Hillestad B.[2], Tinch, A.E.[2], Martin, S.A.M.[3], Robledo D[1] and Houston R.D.[1]

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SUMMARY

Infectious Salmon Anaemia Virus (ISAV) causes a notifiable disease which can require culling of entire stocks when infected fish are identified, and is a major problem for salmon breeders and producers worldwide. Although several vaccines are available, there are barriers to their widespread use. Therefore, selective breeding to produce ISAV-resistant strains of Atlantic salmon (*Salmo salar*) is a high priority for the industry. Genomic selection and potentially genome editing can be applied to enhance host resistance, but these approaches will benefit from improved knowledge of the genetic and functional basis of the trait. In this study we have i) estimated the heritability of resistance to ISAV in a commercial Atlantic salmon population, ii) dissected the genetic architecture of the trait using a genome-wide association study (GWAS), and iii) compared the transcriptomic responses to infection in resistant and susceptible animals.

A total of 1,353 fish from 193 families of the Salmobreed strain (Benchmark Genetics, Norway) taken from an ISAV cohabitation challenge experiment performed at Veso (Norway) were genotyped using a 50 K SNP array. Cumulative mortality reached 23 % and most mortalities occurred between the 20th and 23rd day post infection (dpi). The estimated heritability of resistance to ISAV was 0.21 and 0.26 for binary survival and time to death, respectively. A GWAS revealed a polygenic architecture for resistance, with no genomic region explaining more than a 3 % of the genetic variation. For assessment of functional genomic basis to resistance, 4 resistant and 4 susceptible fish (based on family mortality and genomic breeding values) were selected at each of three timepoints [pre-challenge, 7 and 14 dpi], and RNA samples from heart and head kidney were sequenced. A clear response to ISAV was observed in the heart (4,927 and 2,437 differentially expressed genes, 7 and 14 dpi vs control), however the response in the head kidney was less marked (75 and 41 differential genes, 7 and 14 dpi vs control). There were a relatively small number of differentially expressed genes (DEG) between resistant and susceptible fish (13-18 DEG per timepoint), but these included several interesting innate immune response genes such as interferon-induced very large GTPase 1, E3 ubiquitin/ISG15 ligase TRIM25 (involved in innate immune defence against viruses), or transcription factor Kruppel-like factor 2 (regulates inflammatory processes).

In summary, ISAV resistance has a significant genetic component in Atlantic salmon, albeit with a polygenic architecture. Therefore, this trait is amenable to genomic selection and incremental improvement can be achieved through salmon breeding programmes. We have detected a number of candidate genes that contribute towards understanding the functional basis of resistance to ISAV in Atlantic salmon, and are appropriate targets for downstream functional approaches such as genome editing.

TRANSCRIPTOMIC RESPONSE OF MUSSEL GILLS AGAINST A BATH INFECTION WITH *Vibrio splendidus*

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SUMMARY

Mussels (*Mytilus galloprovincialis*) are filter feeder animals that are constantly in contactwith a wide range of microorganisms, some of which are potentially pathogenic to them.Since it is not yet clearly understood how mussels recognize and respond to pathogens, itmade us focus on the mechanisms that these animals could use in order to deal with a bacterial infection. In order to replicate natural conditions, a bath infection was conductedwith *Vibrio splendidus*.
Mussels were able to totally remove the bacteria from their body and from the tank water.Gills seemed to have a central role in removing and cleaning all the bacteria and antibacterial and antiviral activities were detected in this tissue. A transcriptomic study performed after the bath infection revealed a total number of 1156 differentially expressedgenes. Genes contributing to biological processes such as immune response activation pathways and its regulation with cytokines, cell recognition, adhesion and apoptosis weresignificantly up-regulated under the infection. Among the down-regulated genes, antimicrobial peptides genes such as Mytimicin and Defensin were observed. Long non-coding RNAs were also analyzed revealing expression regulation over response related genes. Gills response against the bath infection was compared with a previous transcriptomic approach on hemocytes against a systemic infection, revealing theirdifferent functions.
Our results suggest that the gills are key players for recognition of pathogens and the activation and regulation of the mussel innate immune response.

IDENTIFICATION OF FUNCTIONAL GENES RELATED TO HOST RESISTANCE TO *Piscirickettsia salmonis* IN ATLANTIC SALMON (*Salmo salar*) USING RNA-SEQ

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SUMMARY

Salmon Rickettsial Syndrome (SRS), caused by the bacterium *Piscirickettsia salmonis*, is one of the most serious infectious diseases affecting Atlantic salmon production. Improvement of host resistance using selective breeding is a potential avenue to tackle SRS. To achieve this, knowledge about the genetic basis of host response and identification of genes and pathways involved in that response is valuable. Therefore, the purpose of this research is to discover functional genes impacting on host resistance to SRS using RNA-Sequencing of head-kidney and liver samples from a large-scale SRS challenge.

2,377 Atlantic salmon (*Salmo salar*) smolts from 104 families were challenged with *P. salmonis* by IP injection during a trial lasting 47 days. Fish were monitored with mortalities collected daily. The traits measured included binary mortality, days to death, weight and length.Head-kidney and liver samples for RNA-seq were obtained from 50 individuals at per-infectionas well as 3 and 9 days post infection (dpi). Differential expression (DE) analyses were performed in R v.3.3.1 using the Bioconductor package DESeq2.

A clear difference in cumulative mortality percentage was observed between families (3.8% – 79.9%). The heritability of resistance using the binary mortality data was 0.45, and a GWAS suggested a polygenic mode of inheritance. A clear and distinct response to the pathogen was observed in both tissues. DE analyses revealed extensive regulation in head-kidney in response to SRS, with 4,835 and 4,562 differentially expressed genes at 3 and 9 dpi respectively, while the values observed on liver transcriptome were lower, with 1,192 and 2,431 differentially expressed genes at 3 and 9 dpi respectively. Enrichment of several KEGG pathways related to immune response was observed in both tissues. Specific pathways identified as crucial for the host immune response to *P. salmonis* and similar pathogens based on a literature search, and key genes were selected for downstream functional studies.

The results highlight genes and pathways that are important in host response to SRS. A list of candidate genes putatively related to disease resistance was obtained by DE analysis of up-regulated genes during infection. Literature review of the biological function of these genes highlighted the most suitable candidates for functional studies, initially using *P. salmonis* challenges of CRISPR/Cas9 knockout cell lines.

RNA-SEQ ANALYSIS OF EUROPEAN SEA BASS (*Dicentrarchuslabrax* L) INFECTED WITH NODAVIRUS REVEALS POWERFUL MODULATION OF THE STRESS RESPONSE

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SUMMARY

Nodavirus, or nervous necrosis virus (NNV), is the causative agent of viral encephalopathy and retinopathy (VER), a severe disease affecting numerous fish species worldwide. European sea bass, a cultured species of great economic importance, is highly susceptible to the disease. To better understand the response of this organism to NNV, we conducted RNA-Seq analysis of the brain and head kidney from experimentally infected and uninfected sea bass juveniles at 24 and 72 h postinfection (hpi). Contrary to what was expected, we observed modest modulation of immune-related genes in the brain, the target organ of this virus, and some of these genes were even downregulated. However, genes involved in the stressresponse showed extremely high modulation. Accordingly, the genes encoding the enzymes implicated in the synthesis of cortisol were almost the only overexpressed genes in the head kidney at 24 hpi. This stress response was attenuated after 72 h in both tissues, and a progressive immune response against the virus was mounted. Moreover, experiments were conducted to determine how stress activation could impact NNV replication. Our results show the complex interplay between viral activity, the stress reaction and the immune response.

PHAGOCYTIC ACTIVITY AND MARKER GENE EXPRESSION OF THE ADHERENT CELLS FROM INTESTINE AND HEAD KIDNEY OF ATLANTIC SALMON

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SUMMARY

Our knowledge of the fish intestinal immune system, especially the phagocytic cells, is rather limited compared to those of mammals. Hence, we have studied the adherent cells isolated from the intestine of Atlantic salmon vis-à-vis those of the head kidney cells, employing flow cytometry and transcriptomics.
Adherent cells from the distal intestine (AIC) and head kidney (AKC, macrophage-like cells) were separated from the harvested cells of healthy Atlantic salmon. Phagocytic activity of the two cell types was compared based on the uptake of *E. coli* BioParticles™ that was assayed employing an imaging flow cytometer; both AIC and AKC had higher phagocytic activity compared to that of whole leucocyte population from the respective tissues. Interestingly, the phagocytic cells from the AIC had different morphology compared to AKC.
RNA-sequencing was performed to compare the transcriptomes of AIC and AKC. Employing the normalized read counts from DESeq2 analyses, we describe the expression of immune cell markers, cytokines, chemokines, mucins and toll-like receptors. The adherent cells from the two organs had apparently higher expression of macrophage-related markers compared to markers of other cell types such as T and B cells. Statistical analyses revealed significant differences between the expressions of the above mentioned immune-related genes in AIC and AKC.
This study provides a snapshot of the overall gene expression profile of the adherent cells from the intestine and head kidney. Our next step is to understand the correlation between target genes and their microRNA to find the potential significance of the mRNA-microRNA interaction in both cells.

PROFILING MICROBIAL COMMUNITIES FOR HEALTHIER OYSTER HATCHERIES

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SUMMARY

The cultivation of Pacific oysters is worth £7.5M per annum to the UK, providing high quality produce to a global market and skilled employment in rural areas. Unlike neighbouring countries in Europe, UK waters remain largely free from the devastating Ostreid herpesvirus (OsHV-1). As such, the majority of UK oyster farmers must source their oysters from disease-free oyster hatcheries in the UK, of which there are currently two. One of these hatcheries has recently experienced significant and sporadic early life mortalities, which it is suspected are associated with microbial contamination. Possible sources of contamination include, for example, the seawater intake (via compromised filtration systems), the adult oysters (simultaneously with gametes) or the algal culture system.

To elucidate the perturbations that may result in a mortality event, we have designed a sampling strategy which captures microbial genomic DNA in the water at all stages of the hatchery process, pre- and post- filtration, in addition to testing the larvae themselves and their environment at various stages of development. Water samples havealso being taken for possible chemical/heavy metal analysis. We aim to capture samples from oyster larvae cohorts with and without major early life mortality events by sampling through multiple spawning cycles. These samples are being sequenced using ametabarcoding approach for prokaryotic and eukaryotic microbes with the Nanopore MinION system. Microbial diversity and abundance will be correlated against metadata, comprising local weather conditions, water quality analysis, hatchery water conditions (temperature, algal content, salinity and pH), in addition to the number of larvae present. Selected interesting samples will be processed for whole genome sequencing to describe the core microbiome of the hatchery and to fully characterise interesting microbes.

To date, we have described microbes associated with normal functioning hatchery water systems and through rarefaction analysis, have identified the optimal sequencing depth required for each sampling point. By continuing to analyse the microbial components of these samples, we will describe variations in the hatchery system, identify its compromised areas, and provide the first atlas of microbes associated with healthy/unhealthy larval culture.

EVALUATION OF IMMUNE-RESPONSE BIOMARKERS IN GILTHEAD SEABREAM FED WITH NOVEL FEED FORMULATIONS

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SUMMARY

The aquaculture industry continues to grow faster than any other food production sector. This growth was also convoyed by increased consumer environmental awareness and knowledge. Hence, the necessity to make aquaculture as sustainable as possible became more obvious day-by-day. This work is part of GAIN project and aims to rise solutions to this need. For that four diets were formulated for in gilthead seabream (*Sparus aurata*) rich in animal protein (PAP), without animal protein (NOPAP) and a combination of diverse emerging ingredients (MIX), against a control diet. The selected ingredients were defined based on circularity principles, maximizing resource efficiency. The immune parameters analyzed (bactericidal, IgM, protease and anti-protease activity) suggest that fish fed with NOPAP diet showed a slight stimulation of innate immunity. This is corroborated by the head kidney gene expression results, which also showed that *il-8* was up-regulated in NOPAP, PAP and MIX groups. The PAP group presented a strong pro-inflammatory profile, evidenced not only by the up regulation of *il-8* but also other cytokines (*il-1β, tnf-α*), chemokines (*ck8*) and chemokine receptors (*ccr3)*. The same pattern was found for the T-cell markers *cd3x, cd4-full* and *cd8a*, whereas the expression of the mucosal *igt-m* was consistently down-regulated. Both MIX- and NOPAP-fed fish showed a more attenuated response, since compared to the control diet, only a reduced number of genes was differentially expressed, *il-8* in NOPAP-fed fish, and *il-8*, *il-1β* and *ck8* in the MIX diet group. These results support the hypothesis that these new formulations are viable options for seabream feed, in particular the NOPAP.

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GENOMIC SELECTION FOR WHITE SPOT SYNDROME VIRUS RESISTANCE IN WHITELEG SHRIMP (*Litopenaeus vannamei*)BOOSTS SURVIVAL UNDER AN EXPERIMENTAL DISEASE CHALLENGE TEST

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SUMMARY

White spot syndrome virus (WSSV) disease is a major problem for shrimp aquaculture throughout the world. The development of resistant shrimp populations is an attractive option for management of WSSV. However, the heritability of WSSV resistance is generally low and genetic improvement by conventional selection has been relatively slow. This study was designed to determine the power and accuracy of genomic selection as a means to improve WSSV resistance in *Litopenaeus vannamei*. Shrimp were experimentally challenged with the disease and resistance was evaluated as dead or alive at a point after infestation. All the individual shrimps in the challenge test were genotyped for 18,643 single nucleotide polymorphisms. Breeder candidates (G_0) were ranked in terms of genomic breeding values for WSSV resistance. Two G_1 populations were produced, one from G_0 breeders with high and the other with low estimated breeding values. A third population was produced from "random" mating of parent stock. WSSV resistance was 15% higher or lower than random selection after one generation of genomic selection for high- or low-WSSV resistance. The average survival was 25% in the low, 38% in the random and 51% in the high-EBV groups. Genomic heritability for the dead or alive trait at 0.41 in the G_1 population was considered to be moderate to high for a such a trait. The realised genetic gain and moderate-high heritability implies that there is large potential to make further genetic improvement for WSSV resistance in *L. vannamei* using genomic selection.

POPULATION GENOMICS AND ADAPTIVE EVOLUTION OF CHINESE SEABASS (*Lateolabrax maculatus)*

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SUMMARY

The marine species usually show high dispersal capabilities accompanied by high levels of gene flow. The gene flow is frequently impeded by environment factors such as temperature, salinity, ocean currents and geographic barriers increasing the genetic and phenotypic differences between populations.Chinese sea bass distributes broad latitudinal gradient spanning from the tropical to the mid-temperate zones. In the past decades, aquaculture of Chinese sea bass has developed fast in south China, but largely rely on germplasm from north China, which creates great uncertainty on wild seabass conservation and sustainable aquaculture. It's eager to perform comprehensive investigation of population studies of Chinese sea bass along the coast of China.

In this case, we performed whole genome sequencing and chromosome-level genome assembly of *L. maculatus*. We also collected numerous genome-with SNP genotypes of sea bass populations along the Chinese coast. After digging deeply into these data, we find out that the most remote two population in the Bohai Gulf and the Beibu Gulf retained significant genetic divergence which are connected by a series of intermediate populations in between. Wealso investigated the potential genetic basis of local adaptation correlating with population differentiation of *L. maculatus*. Genome-scale adaptive micro-evolution analysis identified many functional genes located in regions with significant selective signatures. These genes involve in many biological functions important to adaptation to different local environment, such as acid-base regulation and ion homoeostasis, growth and movement ability. Overall, our genome scale analysis provided insight into population divergence and local adaptation of the highly dispersed Chinese sea bass in the continental margin seas.

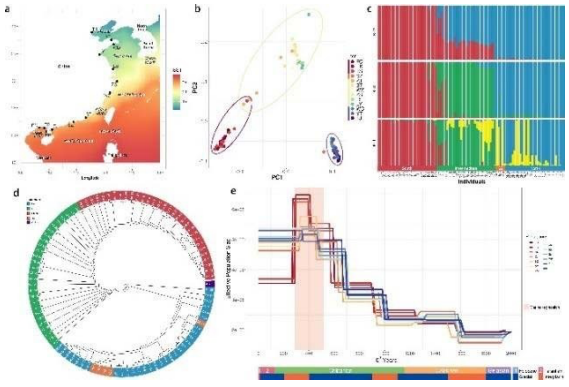

Figure 1. Population structure and effective population size of *L. maculatus*.

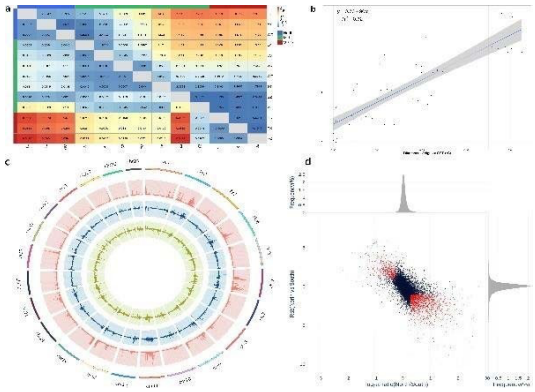

Figure 2. Correlation between genetic distance and SST (a and b). Genome regions with strongpositive selection signals (c and d).

BIOTIC AND ABIOTIC FACTORS SHAPING THE GENOME OF COCKLES (Cerastoderma edule) IN THE ATLANTIC AREA

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SUMMARY

Production of edible cockle (*C. edule*) reached 24,626 tons in 2017 in Europe, being Spain, France and British Isles the main producers. In addition to food supplying, cockles provide many ecosystem services important for coastal areas. Suboptimal management and disease outbreaks, particularly *Marteilia cochillia* parasite, represent serious threatens for cockles in the Atlantic area. Here, using 2b-RADseq we tuned up a set of 9,309 SNPs to analyse the genetic structure of *C. edule* both at macro- and micro-geographic scales, as well as at a temporal scale (across consecutive cohorts). Twenty-eight samples including 746 individuals from 22 locations were studied. The recently assembled cockle genome was used as reference and more than 50% identified SNPs matched to a single genomic position. A practical molecular tool constituted by seven diagnostic SNPs was developed to distinguish edible and lagoon (*C. glaucum*) cockles, easily confounded at juvenile stages, whichconfirmed the pertaining of samples to *C. edule*. No significant genetic differences were detected between consecutive cohorts of 0+ individuals sampled at five locations (10 samples). Gene diversity per population ranged between 0.070 and 0.079 (average 0.074) and, despite a slight heterozygote deficit was systematically observed, none population deviated from panmixia. Pairwise genetic differentiation (F_{ST}) ranged between 0 and 0.067 (global F_{ST} = 0.034) and the Structure software identified two main population units above and below the 48º N parallel in the whole Atlantic Area. However, a refined analysis (DAPC, other likely K values, AMOVA) suggested additional substructuring in the northern (Irish, Wales and North Sea subgroups) and the southern (South Portugal, North Portugal/Spain subgroups) groups, thus totalling five population clusters. These five subgroups could represent a first approach for defining management units. Bayescan analysis identified 579 outlier loci at $P < 0.05$ against the neutral background (~ 5% of the total), mostly relatedto divergent selection. The Structure analysis performed only with those outliers detected again two main northern and southern groups, but other likely K values suggested a much more refined structuring. Three main consistent subgroups were identified in the northern group and a highly admixed constitution was shown in the south, excluding the southern Portuguese populations. Thishigh admixture could be related to movement of stocks related to cockles production. No significant structuring was detected at microgeographic scale in Galicia (NW Spain), although slight evidences of isolation by distance were detected. Twenty-two outliers, all related to divergent selection, were identified in Galician samples, and one of them, separated the marteilia-free from marteilia-infected areas. Ongoing work involving abiotic (temperature, salinity, currents, oceanic fronts) and biotic (parasite, microbiota and non-indigenous species distributions) factors will allow to refine the structure looking for correlation of genetic diversity with environmental variables, and putatively identify candidate genes under selection by mining in the cockles genome.

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BALANCING SELECTION AT THE ATP BINDING SITE OF HEAT SHOCK COGNATE 70 (HSC70) CONTRIBUTES TO INCREASED THERMOTOLERANCE IN *(Artemia franciscana)*

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SUMMARY

A number of single-nucleotide polymorphisms (SNPs) in heat shock protein 70 (HSP70)family genes have been reported to be associated with stress tolerance. In this study, the presence of SNPs in either the *ArHSP70* and *ArHSC70* genes (heat shock cognate gene) was verified in 2 *Artemia franciscana* populations, either a control population (CF12) ora population selectively bred for increased induced thermotolerance (TF12) over 12 generations. In TF12 animals, a novel non-synonymous SNP was identified at position 171 of the cDNA (C171A; N57K; in the ATP/ADP binding site) in *ArHSC70* but notin *ArHSP70*. Cloning and expression of the 2 allelic forms in yeast (*Saccharomyces cerevisiae*) confirmed that yeast cells containing an *Ar*HSC70-N57K plasmid could tolerate higher temperatures than yeast cells containing an *Ar*HSC70-wild type plasmid. This strongly suggests that the SNP at C171A (N57K) increases thermotolerance of the TF12 *A. franciscana* population. Upon analysis of individuals of the CF12 and TF12 populations it appears that the C171A allele frequency increased substantially in the TF12population. Both populations appear not to be in Hardy-Weinberg equilibrium, with CF12displaying an apparent lack of heterozygotes, while the TF12 displays a large excess of heterozygotes. It is argued that these observations are a novel case of balancing selection.As such, HSP genes join a group of immune related genes where balancing selection contributes to population fitness.

OLD AND RECENT INBREEDING EFFECTS ON FEMALE SIZE AND REPRODUCTION TRAITS IN A RAINBOW TROUT SELECTED LINE

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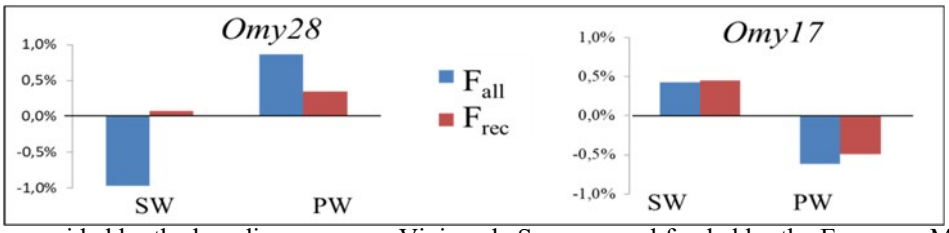
SUMMARY

Selection for production traits in closed and small broodstock populations of rainbow trout over the last 30 years have induced significant levels of inbreeding [1]. Inbreeding can be derived from the identification of homozygous genomic segments, named ROH for run of homozygozity [2]. The size of the ROHs makes it possible to estimate how long there has been inbreeding in the population. From this ROH property, the aim of the study was to assess the age of inbreeding events affecting rainbow trout size and reproduction traits in a French selected line.
We analyzed the performance of 1,366 females under mixed linear animal model including the fixed effects of the two study cohorts and the spawn week within cohort. A first model fitted the pan-genomic inbreeding coefficient as a unique covariate and a second model fitted altogether the chromosomal inbreeding coefficients as 30 covariates explaining performance. For both models, we consider the cumulated effects of inbreeding over all generations (F_{all}) or only the effects of recent inbreeding events (F_{rec}) through ROH sizes longer than 10Mb, which correspond to inbreeding approximatively occurring the 3 last generations. The study traits were the female post-spawning weight (PW), the spawning date (SD), the spawn weight (SW), and the egg average weight (EW).
At the all genome scale, we observed significant effects of inbreeding only for SD and EW, with +10% in F level leading to performance variations of +12.3% and -3.8%, respectively. While both recent and ancient inbreeding effects were significant for SD, only recent inbreeding events affected EW. At the chromosome scale, both recent and ancient events of inbreeding affected all the study traits with negative but also positive effects (Fig1). As largely described in the literature, the main observed effects for all traits were negative impacts of recent inbreeding. However some positive effects of recent inbreeding (see Omy17 for SW) or old inbreeding (see Omy28 for PW) were also observed (Fig1). A unique case of inbreeding depression due to ancient inbreeding events (see Omy28 for SW) was detected (Fig1). To conclude, for all traits, both recent and ancient inbreeding events affect female size and reproduction traits in rainbow trout. Despite a global trend towards inbreeding depression due to recent inbreeding events, positive impacts of local inbreeding are also commonly encountered for all traits. These results shed light on the genetic architecture of inbreeding depression for female size and reproduction traits and its evolution along the genome and over generations.

Fig1. Effect of a +10% increase in inbreeding coefficients for Omy28 and Omy17 on SW and PW performance.



Material was provided by the breeding company Viviers de Sarrance and funded by the European Maritime and Fisheries Fund and FranceAgrimer (SG-Truite project, n° RFEA47 0016 FA 1000016).

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BENEFITS OF GENOMIC EVALUATION IN AQUACULTURE BREEDING PROGRAMS WHEN TANK EFFECTS ARE PRESENT

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SUMMARY

In order to record the pedigree, in aquaculture breeding programs families can be reared in separate tanks until the fish is large enough to be physically tagged. A particular problem with separate rearing of families is that an environmental effect common to the members of the same family (the so called 'tank effect') is introduced. In hierarchical designs, when standard BLUP is used in the genetic evaluation, this effect is difficult to be disentangled from the genetic effect, reducing thus the expected genetic gain. Beside the higher accuracies of estimated genetic effects achieved with genomic evaluation, this technology could lead to a better separation of tank and genetic effects. The objective of this study was to compare, through computer simulations, the selection response achieved when tank effects are present and genomic evaluation is performed for an additive trait with a heritability of 0.4. Three different levels of tank effects were simulated (c^2 = 0.0, 0.1 and 0.3, where c^2 is the proportion of phenotypic variance explained by tank effects). Genomic evaluation (GE) using 3,000 SNP/Morgan, and BLUP were considered. Also, scenarios ignoring or including the tank effectin the evaluation model were compared. Each generation 100 males and 200 females were selected and mated hierarchically (one male with two females) to obtain 200 full-sib families. Two family sizes (n) of 10 and 50 were tested. After ten generations of selection, GE obtainedup to 8% more gain than BLUP for c^2 = 0.0. For c^2 > 0.0 (i.e. 0.1 or 0.3) the advantage of GE over BLUP was up to 10% for n = 10 and up to 25% for n = 50. For both evaluation methods, higher gains were achieved when the tank effect was included in the model than when this effectwas ignored in all scenarios simulated.

GENETIC SELECTION FOR GROWTH DRIVES DIFFERENCES IN INTESTINAL MICROBIOTA COMPOSITION AND PARASITE DISEASE RESISTANCE IN GILTHEAD SEA BREAM

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SUMMARY

The key effects of intestinal microbiota in animal health have led to an increasing interest in manipulating these bacterial populations to improve animal welfare. The aquaculture sector is no exception and in the later years many studies have described these populations in different fish species. However, this is not an easy task, as intestinal microbiota is composed of very dynamic populations that are influenced by different factors, such as diet, environment, host age and genetics. In the current study, we aimed to determine whether the genetic background of gilthead sea bream (*Sparus aurata*) influences the intestinal microbial composition, how these bacterial populations are modulated by dietary changes, and their effect on disease resistance.

To that aim, three different groups of families of gilthead sea bream that were selected during one-two generations for growth (fast, intermediate and slow)[1] were kept together in the same open-flow tanks and fed a control or a well-balanced plant-based diet during nine months. Twelve animals per group were sacrificed and the adherent bacteria from the anterior intestinal portion were collected and immediately used for DNA extraction. The V3-V4 region of the 16S rRNA of each individual sample was amplified and sequenced by Illumina MiSeq. After quality filtering, taxonomic assignment was performed with a custom-made pipeline using the RDP database. Alpha diversity was calculated using Phyloseq and beta diversity using PERMANOVA and PLS-DA models. Metagenome prediction and pathway analysis were performed using Piphillin. In parallel, 30 fish of the fast- and slow-growth groups were infected with the intestinal parasite *Enteromyxum leei* and the disease signs, prevalence, intensity and parasite abundance were evaluated.

No differences were detected in alpha diversity indexes among families, though the bacterial composition was significantly different. Of note, the plant-based diet significantly changed the microbiota in the intermediate- and slow-growth families, with a much lower effect on the fast-growing group. However, the small changes detected in this set of families potentially account for more changes at the metabolic level when compared to the other families. Upon parasitic infection, the fast-growing group showed significantly lower disease signs and parasite intensity and abundance than the slow-growing animals. These results show a clear genome-metagenome interaction indicating that the fast-growing families harbour a microbiota that is more flexible upon dietary changes and can help to cope with intestinal infections.

[1]Perera *et al*., 2019. Aquaculture 507: 349–360.

DOES SELECTIVE BREEDING INFLUENCE THE INTESTINAL BACTERIAL COMPOSITION OF NILE TILAPIA?

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SUMMARY

Selection is a practice in animal farming wherein organisms of a particular trait are chosen to obtain best-suited progeny. Fishes have a high level of phenotypic plasticity and many molecular studies have shown that farmed fish adapt to captivity. Although there are several reports on the effect of domestication on behavior, physiology and morphology of farmed fishes, the influence of anthropogenic selection on the microbiota is yet to be revealed. Hence in the present study, Nile tilapia *(Oreochromis niloticus*) was used as a model organism to study the changes in the microbial community composition of four types of laboratory-reared fish groups. For this, first we obtained eggs from wild-caught female tilapia and transported them to the Research Station, Nord University,Bodø, Norway. The eggs were hatched and reared in separate tanks as different families (F0 generation). Thereafter, they were split into two groups: non-selected (of average weight) and selected (over 10% larger than average) were used to obtain the F1 generation. We examined the effect of selection and family on the microbiota, by collecting samples from the mouth, anteriorand posterior intestine of fish maintained at 28 ºC in the freshwater recirculation system of the research facility. DNA was extracted from these samples. Thereafter, primers 357F (ACTCCTACGGGAGGCAGCAG) and 806 R (GGACTACHVGGGTWTCTAAT) were used to
amplify the V3-V4 regions of the 16S rRNA of the bacterial genomes. PCR products were purified,
quality checked and used for library preparation, following the Illumina 16S amplicon sequencing library preparation protocol. The prepared libraries were sequenced using the Illumina MiSeq. The quality of the reads generated by the sequencer was checked using FasQC and Flexbar software. Next, using the MICCA pipeline the reads were assigned to their respective bacterial operational taxonomic units. Further downstream analyses enabled us to compare the bacterial communities in the digestive tract of the four fish groups.
This study provides insight into the effect of selection for growth on the bacterial communities in the digestive tract of Nile tilapia.
Acknowledgements: This study has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme [grant 683210] and from the Research Council of Norway under the Toppforsk programme [grant 250548/F20].

ANALYSIS OF REPETITIVE SEQUENCES IN THE *Solea senegalensis* GENOME AND THEIR ROLE IN THE EVOLUTION OF A PROTO SEX-CHROMOSOME

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SUMMARY

Repetitive sequences are implicated in chromosomal rearrangements and are responsible for a substantial proportion of the karyotype variability observed in several groups. In addition, they play an essential role in the structural and functional evolution of genomes, particularlyin the sexual chromosomes. The Senegalese sole (Solea senegalensis) is a valuable flatfish in aquaculture albeit few studies have addressed the mapping and characterization of repetitive DNA families.The karyotype of S. senegalensis has been described as 2n = 42 and the largest metacentric chromosome (chromosome 1) is proposed to have evolved through the Robertsonian fusion of two acrocentric ones in S. senegalensis, which has a XX/XY system. We analyzed the Simple Sequence Repeats (SSRs) and Transposable elements (TEs) content from fifty-seven BAC clones (spanning 7.9Mb) of this species, located in chromosomes by multiple fluorescence in situ hybridization (m-BAC-FISH) technique. The SSR analysis revealed an average density of 675.1 loci per Mb and a high abundance (59.69%) of dinucleotide coverage was observed, being 'AC' the most abundant. An SSR-FISH analysis using eleven probes was also carried out and seven of the 11 probes yielded positive signals. Regarding TEs, DNA transposons (Class II) were the most abundant. In Class I, LINEelements were the most abundant and the hAT family was the most represented in Class II. Rex/Babar subfamily, observed in two BAC clones mapping to chromosome pair 1, showed the longest match, highlighting the possible role of the Rex element in the evolution of this chromosome. Besides, a more complete characterization of the chromosome 1 was made (14 BACs) and revealed the presence, in the subcentromeric region, of the highest value for the number of loci of retroelements and for the coverage of simple repeats. Moreover, the presence of a satellite "chromosome Y" (motif length: 860 pb) also was detected in this region. This subcentromeric region contains the dmrt genes, which are usually associated to sex determining in some species, and the presence and accumulation of these repetitive sequences could be indicative of a non- (or low-rate) recombining region and could account the evolution of this putative sex-determining chromosome in S. senegalensis. Funding: This research was funded by the Spanish Ministerio de Ciencia e Innovación MICINN-FEDER (projects RTI2018- 096847-B-C21).

FINE MAPPING OF QTL ASSOCIATED WITH SPONTANEOUS MASCULINISATION IN XX-FEMALE RAINBOW TROUT

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SUMMARY

Rainbow trout (*O. mykiss*) has a male heterogametic (XY) sex-determination system and its sex-determining gene, sdY, has been characterized [1]. Surprisingly, a small proportion of phenotypic males is regularly observed in all-female trout stocks. Previous experimentalstudies evidenced a complex genetic and environmental control of spontaneous maleness [2, 3]. In this study, we aimed at describing the genetic architecture of the trait in a French all- female trout population using GWAS approaches based on medium-throughput genotyping and whole genome sequencing (WGS).

Fish were produced from 5 factorial mating designs (50 sires, 50 dams). Eggs were distributed into two batches incubated at 12°C. At the end of yolk resorption, one batch was exposed to 18°C for 1100 degree-days, covering the expected window of gonad differentiation. The two groups were then reared at the same temperature (12 - 14.5°C). At 10 and 15 months fish (10,000 per batch) were sexed by visual observation of both gonads and distributed into 3 sex classes: female, male and intersex. All males (n=163) and intersex (n=132), and 858 randomly chosen females were genotyped using the 57K SNP Axiom™ Trout Genotyping Array. The genotypes of the 1,139 offspring were imputed to WGS using the genome sequence of the dams. Two GWAS approaches (a marker-by-marker and a Bayesian variable selection method) were performed on 31K SNPs and at the WGS level (8.7 million SNPs).

The overall maleness rate (males + intersex) was 2.0% at 12°C and 0.9% at 18°C. Maleness genomic heritability ranged from 0.48 (± 0.04) to 0.62 (± 0.06) depending on the GWAS approach used. With the 31K genotypes, 3 QTLs associated with maleness were detected on Omy1, 12 and 20. The WGS information allowed to split the QTL on Omy1 into two distinct QTLs. Depending on the analysis, the most significant of those 2 QTLs explained between 4 to 14% of the total genetic variance. Three putative candidate genes were located within this QTL, paving the way towards identification of causal mutation(s) responsible for spontaneous maleness of XX-female rainbow trout.
This work received funds from the European Maritime and Fisheries Fund and FranceAgrimer (NeoBio project, n° R FEA470016FA1000008).
[1] Yano, A., et al., 2012. Current Biology 22(15):1423-1428.
[2] Quillet E., et al., 2002. Journal of Heredity, 93:91–99.
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SINGLE-CELL RNA-SEQ IDENTIFIES HORMONE-PRODUCING CELL TYPES IN THE TELEOST PITUITARY

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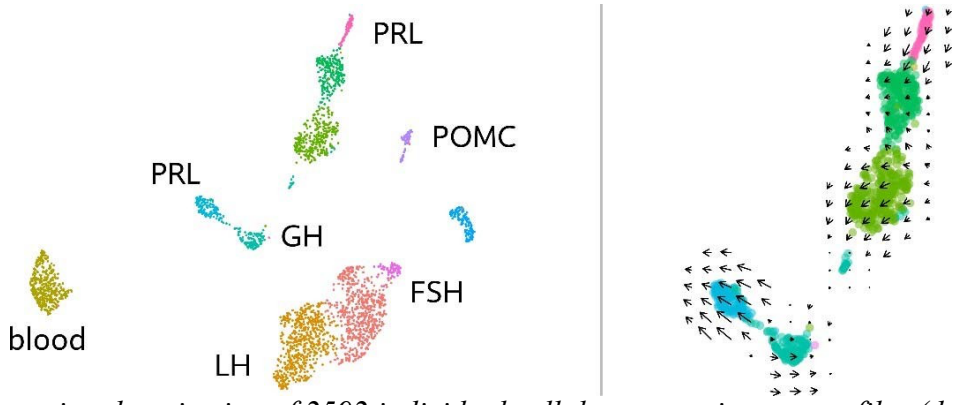
SUMMARY

The pituitary is the master endocrine gland that controls a variety of physiological functions including growth, metabolism, homeostasis, reproduction, and response to stress. These functions are modulated by the secretion of several protein hormones, *e.g.* growth hormone (GH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Unfortunately, key details of hormone production and its regulation are still poorly understood in teleost fish.

In order to elucidate mechanisms at the cellular level, we decided to study the pituitary using single-cell transcriptomics (scRNA-seq). In contrast to regular ('bulk') RNA-seq, this approach yields transcriptomic profiles for individual cells, instead of profiles averaged over many distinct cell types. We have used the 10x Genomics scRNA-seq platform to examine 2592 individual cells from the pituitary of the model teleost medaka (*Oryzias latipes*).

Our single-cell data reveal eight hormone-producing cell types, demonstrating a strict division of labour – each hormone is produced by a dedicated cell type. This contrasts with the tetrapod pituitary, in which a single cell type can produce, for example, both LH and FSH. Many of these cells show extreme specialization: for instance, cells producing proopiomelanocortin (POMC, a precursor for hormones controlling metabolism, pigmentation and stress) devote more than 50% of their transcript pool to the production of this protein. Finally, we identified two novel populations of prolactin-producing cells with different developmental origins. In fish, this hormone is involved in osmoregulation, amongst other functions.

We are using these findings to construct a spatial and functional atlas of the entire teleost pituitary. We expect this will prove to be an essential resource for our studies on reproduction in various fish, including important aquaculture species such as Atlantic salmon.



Two-dimensional projection of 2592 individual cellular transcriptome profiles (dots). Colours represent clusters of cell types, arrows indicate inferred developmental trends in a subset.

ECTOPIC EXPRESSION OF LIPID HOMEOSTASIS GENES IN THE TELEOST PITUITARY GLAND DURING SEXUAL MATURATION

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SUMMARY

Directing both organismal homeostasis and physiological adaptation, the pituitary is a key endocrine gland in all vertebrates. It communicates the needs of the organism to different organs by secreting hormones into the bloodstream. Here, we have used the model fish medaka to investigate developmental dynamics in the pituitary using a comprehensive RNA- seq time series. Using expression trend analyses we show that one of the most prominent changes during sexual maturation is the strong decrease in expression of genes encoding secreted lipid transport proteins, which are typically only produced by the liver. By integrating developmental trends with single-cell transcriptomics, we demonstrate that specific cells are responsible for this expression pattern. With levels as high as for established peptide hormone genes, this ectopic expression exposes a major new mechanism in thejuvenile teleost pituitary. In addition, it implies the existence of unexpected connections between endocrine communication, lipid homeostasis, and sexual maturation.

PERMANENT GONADAL EPIGENETIC CHANGES IN RESPONSE TO HEAT DURING EARLY DEVELOPMENT INZEBRAFISH

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SUMMARY

The environment can influence the epigenome through modifications that alter gene expression and, consequently, can produce the appearance of a new phenotype. DNA methylation and regulatory microRNAs (miRNAs) are two types of epigenetic mechanisms responsible to integrate environmental cues. In fish, epigenetic modifications can follow as result of external inputs, and some can affect sex determination and differentiation. However, the underlying molecular mechanisms remain poorly understood. Currently, the search for epigenetic biomarkers (epimarkers) to identify specific phenotypes is at the forefront of research. The present study aimedto find epimarkers (DNA methylation and miRNAs) in mature fish gonads linked toabnormal thermal conditions that these fish might have suffered during early gonadaldevelopment. Zebrafish (*Danio rerio*) larvae were exposed to either control or hightemperature during sex differentiation (18–32 days post fertilization). DNA methylationin the promoter region of a set of key genes related to sexual development was studiedin adult gonads by a targeted sequencing approach (Multiplex Bisulfite Sequencing, MBS). miRNA expression was explored with RNA-seq using Illumina technology. Furthermore, the spatial distribution of a selection of miRNAs was studied in the ovaries and testes by fluorescent *in situ* hybridization (FISH). Results showed differences in the methylation level of the promoter of some genes between sexes andtemperatures, thus allowing to predict, by machine-learning strategies, epimarkers associated with sex and previous thermal exposure. By biocomputational analysis, weidentified 24 unique miRNAs targeting 402 RNA transcripts and that responded differentially to heat in the gonads. Some of these miRNAs were mostly located to germcells. This study identified DNA methylation and miRNAs changes in the zebrafishadult gonads that can be considered permanent epimarkers of past thermal events. Thiswork was supported by MINECO grants AGL2016-787107-R "Epimark" to FP, AGL2015–73864-JIN "Ambisex" to LR and scholarship BES-2014–069051 to AV. This study identified DNA methylation and miRNAs changes in the zebrafish adult gonads that can be considered permanent epimarkers of past thermal events.
This study identified DNA methylation and miRNAs changes in the zebrafish adult gonads that can be considered permanent epimarkers of past thermal events. Currently, the search for epigenetic biomarkers (epimarkers) to identify specific phenotypes at the forefront of research.

MULTIGENERATIONAL EPIGENETIC INHERITANCEOF MASCULINIZING TEMPERATURES IN THE EUROPEAN SEA BASS

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SUMMARY

In the European sea bass (*Dicentrarchus labrax*) variations in offspring sex ratios exist when reared in normal conditions of temperature for early development (13-16ºC). Evidence shows thatwhen reared at higher temperatures (>17ºC) a proportion of the fish which would develop as females under normal conditions are masculinized. Previous research has shown that the DNA methylation of aromatase, a key gene for sexual development, is affected by temperature. Epigenetics integrates genomic and environmental information to beget the final phenotype. Hence, the changes in DNA methylation in response to high temperature can affect the expression of genes relevant for sexual development. Previously, we studied the gonadal DNA methylation profiles on a panel of seven genes related to sexual development in sea bass exposed at low (LT)and high temperatures (HT). We created four families from four sires and one dam (F0), we exposed larvae to LT and HT and examined the DNA methylation by a targeted sequencing approach in juvenile gonads (F1, one year). The genes most affected by both genetics and environment were *cyp19a1a* and *dmrt1,* with opposite sex-specific methylation and temperature responses. In the current study, we aim to include a larger proportion of genes in the analysis. We created eight families by crossing an external female with eight males among F1 of the first experiment that were exposed at different temperatures; two LT, three HT and 3 HT neomales (NM). Based on phenotypic and genotypic data of the offspring (sire type, body weight, body length and sex tendency estimated from a genomic animal model using 57K SNP genotypes) we selected fish representative of each group: by sex, temperature and sire type. In total, we have produced 130 libraries (110 Gb of sequencing data) by Reduced Representation Bisulfite sequencing technique to analyze the DNA methylome of grandparents' gametes (F0, n=2), sires and dam gametes (F1, n=9) and offspring juvenile gonads (F2, n=119). With this approach and the large dataset produced we aim to understand what is the nature of epigenetic inheritance by the comparison of DNA methylation profiles of sperm of the exposed sires (P) and F1 gonads to isolate imprinted regions that escape genomic reprogramming. We will search for differences in DNA methylation of P sperm and F1 gonads between LT males, HT males and HT neomales to understand what is the contribution of the genotype and of the environment to the DNAmethylation profiles, and to elucidate whether an additive effect of elevated temperature across generations exists. The comparison of the different methylome profiles will reveal the relationships between grandparent, parent-to-offspring, and the conserved epigenetic effect of temperature regardless of the family. Preliminary results show that there is a total of 4,891 and 3,992 differentially methylated cytosines in females and males, respectively, between progeny exposed at LT and HT from sires exposed at LT (F1). Next, we are going to study how are theseresults among the progeny of sires exposed at HT (both males and neomales). Research funded by the European Union's 7th Framework Programme under Grant Agreement 652831 (AQUAEXCEL2020, Transnational Access project AE040073) grant and by the Ministry of Economy and Competitiveness 'Epimark' (AGL2016-78710-R) grant to FP.

NON-CODING RNAs ARE INVOLVED IN THE REGULATION OF TELEOST GONAD MATURATION

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SUMMARY

Wild sharpsnout seabream (*Diplodus puntazzo*) spawn naturally under captivity; however, hatchery-reared individuals fail to spawn. The aim of the present study was to investigate the role of non-coding RNAs in female teleost gonad maturation. Therefore, we collected gonads from wild and hatchery-reared sharpsnout seabream females at the peak of the reproductive season (October, n=3). One part of the gonad was preserved in a formaldehyde solution for histology and another part was kept in RNAlater to investigate small RNAs through high throughput sequencing (Illumina) and subsequent differential expression analysis. Histology of the hatchery-reared fish gonads revealed them to be immature, exhibiting primary oocytes, whereas wild fish gonads were at the vitellogenesis/early ovarian maturation stage. Through Illumina sequencing the average number of reads for each sample obtained after quality and adaptor trimming was 27 million reads. Differential expression analysis resulted in a total of 152 differentially expressed transcripts between farmed and wild females. Principal Component Analysis (PCA) separated the transcripts into two distinct groups according to their maturation stage. Most of the transcripts revealed to be unannotated transcripts (~77%) and miRNA represented only about 7% of the differential expressed reads. In contrast, 15 % were annotated as rRNAs. Of the unannotated transcripts, 11 (~10%) were able to be classified as long non-coding RNAs (lncRNA). Investigated targets of differentially expressedovarian miRNAs were linked to teleost embryonic and larval development in European sea bass (*Dicentrarchus labrax*) and were further investigated in fish larvae from the flexion until the metamorphosis stage. In conclusion, in the present study, micro RNAs, as well as ribosomal and lncRNAs, are shown to play an important role during female gonad maturation,stressing their involvement in the reproductive biology and early development of teleost fish.

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 727315 (MedAID). Call: SFS-23-2016 Improving the technical performance of the Mediterranean aquaculture

IDENTIFICATION OF CONSERVED SEX-SPECIFIC MICRORNASIN TELEOST SPECIES AND THEIR FUNCTIONALITY IN ZEBRAFISH GONADS

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SUMMARY

Micro RNAs (miRNAs) are small, non-coding RNAs that are involved in post- transcriptional gene regulation in many cellular functions and are conserved throughout evolution. In teleost fish species, miRNAs are believed to play a role in the reproductive system. Understanding the different expression patterns of miRNAs between females and males will help provide insight in the epigenetic events occurring in the gonads, but also develop epimarkers to improve aquaculture production. The aim of this study was identifying miRNAs that are conserved in the ovaries and in the testes of several teleost species. Further, target genes with which miRNAs interact were identified. Available gonadal miRNA data from five different species (Atlantic cod, catfish, Nile tilapia, European sea bass and zebrafish) were obtained together with unpublished zebrafish miRNA data from our lab. Approximately, 3000 published sequences were used for comparisons. Normalized differentially expressed (normalized reads>100) miRNAs were selected among all the available data. Comparison results showed that 31 miRNAs were conserved in all species in both gonads. Additionally, five ovary-specific and five testis- specific miRNAs were identified. Target genes of those 10 sex-specific conserved miRNAs was performed by *in silico* analysis. Using the MiRanda algorithm (score > 140, energy < - 25) and biomaRt package in R, between 9 and 118 genes were identified as potential targets per each miRNA. To confirm target interactions and functionality of the miRNAs in the gonads, *in vitro* gonadal studies in zebrafish with miRNA inhibitors and mimics have started. Studying the *in vitro* functionality of the conserved sex-specific miRNAs will provide insights in the molecular mechanisms behind these miRNAs and their role in the fish gonads. Here we have identified miRNAs that are specific for the ovaries or the testes in several teleost species, some very important for aquaculture. Thus, identified miRNAsthat are conserved throughout evolution and can help in the development of universal epimarkers to improve fish productivity.

EXPLORING EARLY DEVELOPMENT OF A MODEL AND AN ECONOMICALLY IMPORTANT SPECIES THROUGH miRNA's EXPRESSION

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SUMMARY

Embryogenesis includes a cascade of different important processes, such as diversity, morphogenesis, and reproduction. Thus this period is defined as one of the most critical for all organisms' lifetime. The activation -or deactivation- of genes involved in developmental processes is under the strict supervision of regulative mechanisms. One negative regulative mechanism is performed by a group of small non-coding RNA molecules, the miRNAs. First reports of miRNAs described them as significant participants in developmental timing. Since then development has been one of the most studied biological functions being controlled by miRNA regulative mechanism.

Herein the expression of miRNAs during the early developmental stages of a model (three-spined stickleback, *Gasterosteus aculeatus*) and an economically important teleost (European sea bass, *Dicentrarchus labrax*) was investigated. Therefore, miRNA libraries from different developmental stages ranging from the morula stage until 24hph were sequenced applying high throughput Illumina next-generation sequencing technology.

Around three and eight million reads per developmental stage were generated in three-spined stickleback and European sea bass respectively. The generated results suggested that early stages comprise only a small set of miRNAs and are expressed in low amounts, while during the last studied stages both miRNA diversity and miRNA read number increased. Furthermore, and in agreement with the fact that the different developmental stages arecharacterized by different biological functions, qualitative differences in the miRNA expression profile were detected. Utmost example of the above is the case of miRNAs knownas major regulators of stage-specific processes which were identified also in this study as predominantly expressed at the corresponding stages.

TARGETING THE MILD-HYPOXIA DRIVING FORCE FOR METABOLIC AND MUSCLE TRANSCRIPTIONAL REPROGRAMMING OF GILTHEAD SEA BREAM (*Sparus aurata*) JUVENILES

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SUMMARY

Hypoxia is a common stressor in aquatic environments, and fish reduce feed intake and reorganize its metabolism to limit the tissue O_2 demand. This allows to preserve aerobic metabolism by means of a restricted mitochondrial respiration and a shift in substrate preferences as part of the adaptive response of the skeletal muscle to hypoxia exposure. Herein, we aimed to underline new insights on the mild-hypoxia driving force for metabolic and muscle transcriptional reprogramming of gilthead sea bream juveniles. For this purpose, on-growing juveniles of gilthead sea bream were acclimated for 45 days to mild-hypoxia (M- HYP, 40-60% O_2 saturation), whereas normoxic fish (85-90% O_2 saturation) constituted two different groups depending if they were fed to visual satiety (N; control fish) or pair-fed to M-HYP fish (N-PF). Following the hypoxia conditioning period, all fish were maintained in normoxia and continued to be fed until visual satiation for 3 weeks. The time course of hypoxia-induced changes was assessed by changes in blood metabolic landmarks and muscle transcriptomics before and after exhaustive exercise in a swim tunnel respirometer. Maximumfeed intake was reduced by M-HYP pre-conditioning, and both N-PF and M-HYP experienced an improved feed conversion during the normoxia recovery period. M-HYP conditioning reduced circulating levels of free fatty acids and lactate as part of the hypo- metabolic response to face a reduced O_2 availability. In exercise tests, M-HYP group showeda higher critical swimming speed (U_{crit}) that was preserved along the normoxia recovery period. Changes of circulating metabolites and hormones evidenced an enhanced aerobic ATPproduction in M-HYP fish at the end of the conditioning period, whereas anaerobic metabolism was primed at the end of the normoxia recovery period. Heatmap clustering of muscle differentially expressed (DE) genes, after filtering by ANOVA ($P < 0.05$) and PLS- DA (VIP > 1), grouped together N-PF and M-HYP after the mild-hypoxia conditioning. This yielded 222 differentially expressed genes that were increased up to 421 after exercise exhaustion, although this divergent expression pattern was reduced thereafter to 180 genes at the end of the normoxia recovery period. Gene enrichment analysis and protein interaction plots also highlighted a higher contribution of lipid metabolism and, thus, of aerobic metabolism to whole energy supply, shifting towards a higher anaerobic fitness following normoxia restoration. Despite of these changes in substrate preference, M-HYP fish shared a persistent improvement of swimming performance with a higher critical speed at exercise exhaustion. The machinery of muscle contraction and protein synthesis and breakdown was also largely altered by mild-hypoxia conditioning, contributing this metabolic re-adjustmentto the positive regulation of locomotion and to the catch-up growth response during the normoxia recovery period. Altogether, these results reinforce the large phenotypic plasticityof gilthead sea bream, becoming mild-hypoxia a promising prophylactic measure beforepredictable stressful events.

SEASONAL BROODSTOCK MANAGEMENT INFLUENCES THE NUTRITIONAL STATUS, GENE EXPRESSION AND EPIGENETIC GENE REGULATION IN ATLANTIC SALMON PROGENY

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SUMMARY

The 1C nutrients, which include vitamin B6, vitamin B12, and folate, as well as the amino acid methionine, have previously been shown to influence lipid metabolism and epigenetic gene regulation in zebrafish progeny. In the salmon aquaculture industry, the broodstock females are manipulated to spawn both earlier and later than the normal spawning season in order to produce available offspring throughout the year. Maturation and spawning times for salmon can be controlled by regulation of feeding, light and temperature regimes, while the normal spawning season for salmon is in October-November. We therefore analysed whether nutrient status in offspring was affected by seasonal spawning, which may affect growth via epigeneticregulation of gene expression, such as DNA methylation. We specifically studied whether advanced (early) or delayed (late) spawning seasons affect the nutrient status of 1-C nutrients, free amino acids and lipid classes in the muscle and liver of brood fish, and whether these nutritional variations act as modulators of early nutritional based programming by affecting thenutritional profile of newly fertilized eggs as well as early embryo development. To study the long-term mechanisms that are affected by nutrient variations in the embryos, we have studiedboth DNA methylation and RNA sequencing of liver of first feeding larvae. Both advanced anddelayed spawning seasons significantly affected the gene expression in the liver of genes that regulate cell cycle as well as steroid biosynthesis pathway compared to normal spawning season. Particularly interesting is that regulation of the cell cycle signaling pathway, where 33and 28 genes were downregulated in early and late versus normal spawning seasons, respectively; this suggests that spawning season manipulation may affect the growth potential of the larvae. DNA methylation in the liver cells was also affected by manipulation of spawning seasons. Our differential methylation analysis identified over 2000 significantly differentially methylated CpG sites with spawning season, and some of them appeared to associate with over10 KEGG pathways, such as protein processing and cell signaling. The results describe nutritional differences in broodstock, which affect nutrient status in offspring. DNA methylation differences as well as differences in gene expression show that seasonal broodstockmanagement has a major impact on offspring growth, nutrient status and gene regulation. Whenthe nutrition-related impact occurs at early life stages, the focus on broodstock feeding regimesand feed quality can potentially be optimized, and thus programmed for better growth and health in later stages of ontogeny.

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DIFFERENCES IN LIVER DNA HYDROXYMETHYLATION REFLECT THE GROWTH PHENOTYPE

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SUMMARY

Faced with ocean warming and acidification, adapting new fish species to industrial aquaculture is necessary to ensure stable food production. In that regard, the industrial domestication of new fish species can be a long process, therefore identifying key molecular mechanisms that are involved with the earliest genome-wide responses in captivity can contribute towards its acceleration. To test whether epigenetic modifications are associated with unique phenotypes during the early stages of domestication, we used the reduced representation hydroxymethylation profiling (RRHP) method and compared the genome-wide DNA hydroxymethylation (5hmC) profiles in liver from large and small second-generation, full-sibling Nile tilapia (*Oreochromis niloticus*) females. In total, we identified 2457 differentially hydroxymethylated cytosines (DhmCs) between large and small fish groups. Gene enrichment analysis showed that hyper-DhmCs in large fish were located within genes involved in extracellular matrix organization, developmental growth, skeletal system development, as well as the PI3K-Akt, cGMP-PKG and Ras protein signal transduction pathways. These latter pathways are important in cellular signal transduction, cell cycle, proliferation and growth as well as mitochondrial ATP synthesis efficiency (Fig. 1). These findings suggest that 5hmC is involved in regulating major growth-related pathways and that differential 5hmC within these pathways underlies different growth phenotypes that arise during fish domestication. Identifying such key epigenetic markers could potentially result in a faster and more robust selective breeding for future fish domestication programs.

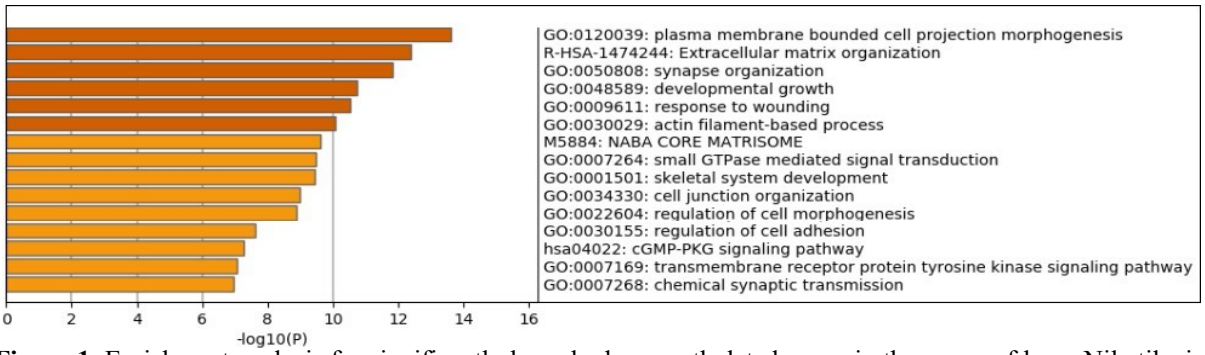

Figure 1. Enrichment analysis for significantly hyperhydroxymethylated genes in the group of large Nile tilapia compared to their smaller counterparts. The y-axis represents the enriched gene ontology (GO) terms and pathways and the x-axis the significance level of each term.

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DIFFERENT TRANSCRIPTOMIC STRATEGIES TO COPE WITH SALINITY CHALLENGE IN NILE AND MOZAMBIQUE TILAPIAS RESPOND TO SYMPATRIC SPECIATION

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SUMMARY

The distribution of aquatic species is determined by several physical factors such as salinity. The salinity gradient between fresh and sea water environments increase the alternatives for the propagation of species and each saline concentration can provide selective pressure for efficient metabolic pathways to cope with the osmotic stress. Freshwater species could diversify from euryhaline ancestors through processes such as landlocking. Adaptation to low salinity content requires uptake and retain ions through the gills and the gastrointestinal tract. Cichlids are a broad range of species distributed throughout the tropical and subtropical areas of Africa and America, manyof them with various euryhaline capacities. That is the case for the Nile tilapia that have moderate salinity tolerance and its sister species Mozambique tilapia that is highly salinity tolerant. We analysed the expressed genes of the gill epithelia in order to determine the transcriptomic architecturein the two species after 6 weeks exposure to salty water. Our results suggest that both tilapias triggerimmune and cell stress responses as well as epithelium turnover for coping with salinity by differential expression of dozens of genes. However, the responsive genes in each species are different both in salty and fresh water, thus illustrating the transcriptomic architecture of the salinity tolerancein each species as a trait characteristic of speciation in sympatry developed after ecological divergence.

EXOGENOUSLY GENERATED REACTIVE OXYGEN SPECIESALTER THE TRANSCRIPTOMIC LANDSCAPE OF ATLANTIC SALMON OLFACTORY MUCOSA

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SUMMARY

The mucosal surfaces are considered the first line of defence in fish and these structures are perpetually exposed to several environmental pressures – from biological insults, *i.e.* bacteria, viruses and parasites, to physical challenges related to farming. Though responses to different environmental stimuli of the major mucosal organs such as skin, gills and gut have been relatively documented, little is known with the responses of the olfactory mucosa. Besides its chemosensory function, the olfactory system plays a vital immunological role to waterborne stimuli.

Reactive oxygen species (ROS) are a number of reactive molecules and free radicals derived from molecular oxygen and are generated as by-products during mitochondrial electron transport. Other ROS molecules may derive from the immediate environment. In farmed fish, these ROS may come from oxidative chemotherapeutants used to address ectoparasitic infections and/or for routine water treatment in recirculating systems. Excessive levels of these environmental ROS may trigger oxidative stress, and if fish are not able to mount an appropriate adaptive response, may lead to detrimental physiological consequences. The impacts of these radicals on the mucosa remains barely explored in fish, especially in the olfactory mucosa.

We identified the molecular signatures associated with the response of Atlantic salmon olfactory rosette to environmental ROS, which was generated by exposing smolts to peracetic acid (PAA), a peroxygen compound. In trial 1, salmon were exposed to 10 ppm PAA for 30 mins every 15 days, and there were three exposures in total. In trial 2, salmon reared in brackishwater RAS were exposed to 1 ppm PAA every three days for 45 days. This presentation will discuss the changes in the transcriptome of the olfactory rosettes and how these alterations play a crucial role in the adaptive processes of salmon mucosa to repeated ROS exposures.

THE MITOCHONDRIAL 5-METHYLCYTOSINE PROFILE IN NILE TILAPIA (*Oreochromis niloticus*)

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SUMMARY

Growth and metabolism are closely linked to mitochondrion, since this organelle's primary function is generation of cellular energy by production of ATP (adenosine triphosphate). Mitochondrion has a compact circular genome (16,5 Kb in average), which is related to its bacterial origin. The mitogenome in Nile tilapia (*Oreochromis niloticus*) is 16,625 bp long and contains 13 protein-coding genes (involved in oxidative phosphorylation), two rRNA genes, 22 tRNA genes and the D-loop (putative control region). The number and the order of mitochondrial genes in Nile tilapia matches those in other cichlids. DNA methylation is one of several epigenetic regulatory mechanisms that control gene expression. In fish, epigenetic changes associated with mtDNA and their potential relation to functional effects on growth is still a question that remains unanswered. In this study, we isolated mtDNA from liver of 5 full-sib Nile tilapia females. A whole genome bisulfite sequencing (Illumina NextSeq) approach was used for identifying methylation patterns within CHH, CHG and CpG context in the mitochondrial genome. We described and compared methylation rate among mitochondrial genes to create the first methylome map of mtDNA in fish. Our results demonstrated the presence of mtDNA methylation in fish, predominantly within non-CpG context. We found a strand-specific distribution of mtDNA methylation, where most of methylated cytosines were located on the light (minus) strand. The D-loop region had the highest mean methylation value among all mitochondrial genes. These data provide a new insight into mitochondrial epigenetics in fish and can serve as a background for further functional investigation of potential epigenetic markers involved in shaping metabolic flexibility and growth in Nile tilapia.

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APPLIED HOLOGENOMICS: LEVERAGING MICROBIOTA SERVICES THROUGH HOLO-OMIC ANALYSES IN FARMED FISH

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SUMMARY

Emerging evidence across all areas of life has revealed the evolutionary importance of the intimate biological interactions between animals and their associated microbiota. Both the host genotype and the host microbiota have been shown to influence host phenotypes, such as growth and disease states. The hologenome concept maintains that the host genome and the host microbial metagenome are subject to essential biological interactions; thus, both should be considered simultaneously as a single interconnected 'holobiont system' when investigating how animals respond to e.g., diet and disease. Based on challenges in aquaculture, we leverage current knowledge in molecular biology and host microbiota interactions to propose an applied holo-omic framework1 that integrates molecular data including (meta)genomes, (meta)transcriptomes, epigenomes, and (meta)metabolomes for analyzing fish and their associated gut microbiota as interconnected and coregulated holobiont systems. I will present data from a suite of ongoing projects - including HoloFish2 and HoloFood3 - that all apply our holo-omic framework to understand the essential molecular interactions by which the gut microbiota shapes phenotypic traits in both Atlantic salmon and rainbow trout. In particular, we look at traits related to growth, novel feed additives, and response to a pathogenic bacterium. We discuss the feasibility and potential of using our holo-omic framework to combine large -omics data sets for more coherent analyses of host – microbiota systems to help steer a more sustainable growth of aquaculture.

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INTESTINAL TRANSCRIPTOME OF ZEBRAFISH FED SOYBEAN AND YEAST β-GLUCAN

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SUMMARY

Soybean contains antinutritional components that can jeopardize the functions of the intestinal barrier. In the present study, juvenile zebrafish were fed three experimental diets for 30 days to understand the effects of dietary soybean. A reference zebrafish diet served as the control while a plant-based diet that had 50% soybean meal was intended to study how this feed ingredient caused changes in the intestine. A third diet supplemented with yeast β-glucan was used to investigate its intestinal inflammation-countering effect. We employed RNA-Seq to observe the changes in the intestinal transcriptome. Soybean meal affected the expression of genes related to intestinal barrier function, metabolism, immune functions, cell cycle, DNA damage and DNA repair. The plausible ability of yeast β-glucan to regulate immune responses and barrier integrity is also described in our study. We have obtained evidence of subdued inflammation in juvenile zebrafish intestine caused by soybean meal and the potential of β-glucan in controlling it.

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DIETARY GLUCANS CAN RESTORE NORMAL LIPID METABOLISM IN ZEBRAFISH

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SUMMARY

Defective lipid metabolism can lead to the development of cardiovascular diseases in humans but dietary interventions can help rectify such defects. It is also known that natural bioactive compounds are effective to reinstate the normal lipid metabolism. Employing the zebrafish model, we have examined the efficacy of dietary oat glucans to alleviate the issues linked to lipid metabolism. We studied the intestinal transcriptome and plasma cholesterol levels in zebrafish, and the results revealed the ability of dietary glucans to restore the circulating cholesterol. RNA-Seq revealed the suppression of endoplasmic-reticulum-associated protein degradation by dietary glucans. We also evaluated the intestinal transcriptome of zebrafish fed the hypocholesterolemic drug, simvastatin; the findings revealed that the drug suppressed pathways linked to amino acid metabolism. Our study sheds light on the possible mechanisms by which glucans and simvastatin alter the intestinal transcriptome of the zebrafish hypercholesterolemia model.

SPONSOR TALK

APPLICATION OF MOLECULAR BIOMARKERS TO FISH NUTRITION

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