immunostimulation, as evidenced by high levels of expression of the genes involved in pathogen recognition, immune signaling pathways, apoptosis and effectors such as protease inhibitors, mucins and perforin-2. In contrast, larvae exposed to the bacterial pathogen *Vibrio coralliilyticus* RE22 showed evidence of immunosuppression. Transcriptome analysis of lines of oysters with varying levels of resistance to the bacterial pathogen Aliiroseovarius crassostreae and the protozoan parasite *Perkinsus marinus* yielded information that confirmed the importance of serine protease inhibitors and other immune molecules in disease resistance. Finally, viral, bacterial, and parasite genomes deposited in genetic databases were indexed and used as a reference for the assembly of reads derived from oyster DNA resequencing and transcriptome (RNA) analysis projects. The coverage and expression of these microbial genomes was calculated and compared between samples. Analysis of resequencing data from 96 oysters collected at sites from Texas to Maine for the presence of known (and unknown) parasites of oyster show that genomic data could be used to mine for information on pathogen abundance and distribution. These experiments illustrate the potential of −omic tools to inform host-pathogen interactions in bivalves.

Keywords: bivalves, probiotic, protozoa, transcriptome, vibrio

Corresponding author.
E-mail address: gomezchi@uri.edu (M. Gomez-Chiarri).

O-113.

Changes in the host microbiota structure and diversity during the immune response of the scallop *Argopecten purpuratus*

K. Muñoz [1,], P. Flores-Herrera [1,*], A.T. Gonçalves [2], C. Rojas [3], C. Yañez [3], K. Brokordt [4], P. Schmitt [1,#].*

[1] Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de, Valparaíso
[2] Laboratorio de Biotecnología y Genomica Acuícola − Centro Interdisciplinario para la Investigación Acuícola (INCAR), Universidad de Concepción, Concepción, Chile
[3] Laboratorio de Microbiología, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[4] Laboratory of Marine Physiology and Genetics (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Universidad Católica del Norte

Abstract
Traditionally it has been considered that invertebrates depend mainly on innate immunity mechanisms to defend against pathogens. However, new evidence suggests that the immune capacity of organisms is related with the symbiotic microorganisms. Indeed, all organisms live in close association with a variety of microorganisms, called microbiota, and several studies support a fundamental role of the composition of this microbiota in the health and homeostasis of vertebrates and invertebrates. In this context, the aim of this work was to determine the microbiota composition of the scallop *Argopecten purpuratus,* one of the most important reared bivalve species in northern Chile and Peru, and to assess changes in the scallop bacterial microbiota during the host immune response. For this, adult scallops were immune stimulated by an injection of heat-killed *Vibrio splendidus* and collected 24 h and 48 h after for analysis. The activation of the host immune response was established by the transcript overexpression of several scallop immune response genes in hemocytes and gills, and confirmed by the protein detection of the antimicrobial peptide big defensin in gills of *Vibrio*-injected scallops at 24 h post challenge. Next, the microbiota structure and diversity from pools of scallops were characterized using 16S rDNA deep amplicon sequencing. Results revealed that the injection of *A. purpuratus* with either filtered sea water or *Vibrio* resulted in a noticeable reduction of the order Bacteroidales and an increase in Mycoplasmatales, Clostridiales, Chlamydiales, among others when compared to non-injected scallops. Notably, particular shifts of some bacterial groups were observed between filtered sea water or *Vibrio* injected scallops, both at 24 and 48 hours. Thus, an overall modulation of the microbiota abundance and diversity according the scallop immune status was detected, allowing the prediction of some changes in the functionality of the microbial community. Finally, DGGE analysis was performed to identify any changes in the scallop microbiota at the individual level. Dendrograms constructed from the single scallop DGGE profiles demonstrate that the changes in the predominant bacterial groups are consistently detected according the immune status of scallops and evident changes occurs 48 hours after the immune challenge. Overall, the present study showed that changes in the structure and diversity of the bacterial communities associated to the scallop *Argopecten purpuratus* are detected after the activation of the host immune response. Now, the relevance of the disruption of the microbial balance in the immune capacity of the host remain to be elucidated. WORK FUNDED BY FONDECYT 11150009.

Keywords: Host-microbiota interactions; scallop; innate immune response; antimicrobial effectors; metagenome

Corresponding author.
E-mail address: paulina.schmitt@pucv.cl (P. Schmitt).
* These authors have contributed equally to this work.

O-114.

Proteomic profile and proteases characterisation of greater amberjack skin mucus after *Neobenedenia girellae* infection

A. Fernández-Montero [1,#,], S. Torrecillas [1,*], F. Acosta [1,*], M.J. Prieto-Álamo [2,*], J. Jurado [2,*], D. Montero [1,*].*

[1] Grupo de Investigación en Acuicultura. Ecoaqua institute. University of Las Palmas de Gran Canaria, Gran Canaria, Spain
[2] Department of Biochemistry and Molecular Biology, Agrifood Campus of International Excellence (ceiA3), University of Córdoba, Severo Ochoa Building, Rabanales Campus, 14071 Córdoba, Spain

Abstract
Skin mucus is known for being the first physical and immunological barrier in fish. Skin mucus is composed by a wide range of proteins, like glycoproteins, structural proteins, metabolic proteins and immune related protein-components. Likewise, proteome changes in Atlantic salmon (*Salmo salar*) have been observed due to sea lice infections, demonstrating the importance of protein-immune defenses against ectoparasite infections. Nowadays, greater amberjack (*Seriola dumerili*) pass-through a biosanitary bottleneck on its on-growing period related with monogenean ectoparasites, which could cause a 90% of mortality (Ogawa et al.,1998). For that reason, this study aimed to compare skin mucus proteome of non-infected and experimentally infected greater amberjack juveniles with *Neobenedenia girellae*, as well as to characterize proteases of this skin mucus. Thirty greater amberjack juveniles of 150 ±12 g were randomly distributed in 3 cylindroconical tanks of 500 liters. After 10 days of acclimation, skin mucus of non-infected fish was obtained, pooled and immediately freeze in liquid nitrogen. Cohabitation with *N.girellae* was conducted with 3 previously infected fish stored in cages for 15 days, when all the experimental fish were infected, skin mucus was sampled. The integrative proteomic approach was conducted using a label-free procedure as LC-MS/MS with a 2-DE-PMF-MS/MS. Protease activity was conducted using azocasein hydrolysis assay, while protease characterization was determined combining azocasein hydrolysis with inhibitors of metalloproteases and serine proteases. Results obtained with LC-MS/MS showed the first microbiota analyses in greater amberjack skin mucus, were the most abundant species belonged to gamma-proteobacteria group, and infected and non-infected fish bacterial presence only differed in 6 genus of bacteria.2-DE-PMF-MS/MS analyses showed differences in proteome profile at a qualitative level. Proteins of p/ 5 and molecular weight ranging between 36-66 KD, typically identified as structure proteins, were clearly affected by degradation for *N.girellae* infected fish. Protease activity analysis showed no difference among infected and non-

infected fish, however proteases populations differed in metalloproteases and serine proteases when comparing infected and noninfected fish.
Keywords: Greater amberjack, Mucus, Skin, Proteomic, Ectoparasites

Corresponding author.
E-mail address: alvaro.montero@ulpgc.es (A. Fernández-Montero).
* These authors have contributed equally to this work.

O-115.

Comparative transcriptome analysis of pilchard orthomyxovirus (POMV) and infectious salmon anaemia virus (ISAV)

F. Samsing [1], J. Hoad [2], P. Mohr [2], M. Dearnley [2], J.W. Wynne [1,#].

[1] CSIRO Agriculture and Food, Aquaculture program, Hobart, Tasmania, Australia
[2] CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia

Abstract
Pilchard orthomyxovirus (POMV) is an emerging virus of concern to the Tasmanian
Atlantic salmon industry. Originally isolated from pilchards in South Australia in 1998, this virus has now caused several high mortality events in Tasmanian farmed Atlantic salmon. Despite its classification as an orthomyxovirus, POMV isphylogenetically divergent from ISAV. While previous research has produced a formal case definition for clinical POMV, the molecular events that underpin viral infection have not been characterized. To this end we have undertaken a comparative transcriptome analysis of the response of Atlantic salmon kidney cells (ASK) to both POMV and ISAV. Despite their genetic divergence, both orthomyxoviruses induced significant, and in some cases similar, innate antiviral responses. Early up-regulation of the host pathogen recognition receptors, RIG-I and TLR3, was observed in response to both viruses and triggered downstream interferon responses. Analysis of transcription factor binding sites in the up-regulated gene sets revealed that the host response to both viruses was largely driven by interferon regulatory factor 1 and 2. Unique host responses were also observed for each virus which are likely a consequence of virus divergence. The potential to exploit these early host response genes as subclinical biomarkers specific to POMV will be discussed.
Keywords: Orthomyxovirus, transcriptome, interferon, biomarker, host-pathogen interaction

Corresponding author.
E-mail address: james.wynne@csiro.au (J.W. Wynne).

O-116.

Characterization of flounder (*Paralichthys olivaceus*) CD4+ T lymphocyte subsets in response to Th-type antigens

Hongfei Tian [1], Jing Xing [1,2,#], Xiaoqian Tang [1], Xiuzhen Sheng [1], Wenbin Zhan [1,2].

[1] Laboratory of Pathology and Immunology of Aquatic Animals, KLMME, Ocean University of China, Qingdao, 266003, PR China
[2] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, No. 1 Wenhai Road, Aoshanwei Town, Qingdao, China

Abstract
The CD4+ T lymphocytes play crucial roles in the adaptive immune system. Naive CD4+ Th cells differentiate into a variety of effector T lymphocytes subsets, such as Th1, Th2, Th17 and regulatory (Treg) cells. These CD4+ T cells widely involved in immune regulation, immune pathogenesis and host defence through subsequent secretion of effector and regulatory cytokines. Two CD4 homologues have been reported in flounder (*Paralichthys olivaceus*), one is like mammalian CD4 molecules, containing four extracellular Ig-like domains, named as CD4-1, and the other is a CD4-like molecule, containing two or three Ig-like domains, termed CD4-2. In this study, identification of CD4-1+ and CD4-2+ T lymphocytes subsets and the immune response to Th-type antigens in flounder were investigated. The epitopes peptides of CD4-1 and CD4-2 molecule were screened with high hydrophilicity, accessibility, flexibility, antigenicity and specificity. Two peptides were synthesized and immunized to the mouse, and then the monoclonal antibodies (mAbs) against flounder CD4-1 and CD4-2 were produced, respectively. The mAbs had high specificity in identifying flounder CD4-1+ and CD4-2+ T lymphocytes subsets. And then, three Th-type antigens, poly (I:C), PMA and β-glucan, were injected to flounder, respectively, the percentages of CD4-1+ and CD4-2+ T lymphocytes and the transcription factors and cytokines in sorted CD4+ cells subsets were detected. The results showed, CD4-1+ and CD4-2+ cells in peripheral blood, spleen and head kidney were all increased after stimulation. Notably, CD4-2+ cells were give stronger response to poly (I:C), which indicated that CD4-2+ cells may play a main role in the Th1-related immune responses. While the proliferation of CD4-1+ cells were showed no difference to three antigens. The Th cells transcription factors and related cytokines in sorted CD4+ cells were sharply up-regulated. These results demonstrate that the CD4+ cells in flounder have potentials to differentiate into different Th cells similar to mammalian.
Keywords: CD4+ T lymphocytes; monoclonal antibody; antigens; immune response

Corresponding author.
E-mail address: xingjing@ouc.edu.cn (J. Xing).

O-117.

Functional additives in low fish meal and fish oil based diets for European sea bass (*Dicentrarchus labrax*): Effects on immune response, stress and disease resistance

A. Serradell [1,#,], S. Torrecillas [1,*], A. Makol [2,*], F. Acosta [1,*], V. Valdenegro [3,*], D. Montero [1,*].*

[1] Grupo de Investigación en Acuicultura. Instituto Ecoaqua.Universidad de Las Palmas de Gran Canaria, Gran Canaria, Spain
[2] Delacon Biotechnik GmbH, Weissenwolffstrasse 14, 4221 Steyregg, Austria
[3] Biomar A/S. BioMar AS, POB 1282 Sluppen, N-7462, Trondheim, Norway

Abstract
The use of terrestrial raw materials to replace fish meals and oils in fish diets may affect fish growth performance and health. In the last years functional additives have been profiled as good candidates to reduce the effects on health and disease resistance derived from this replacement, via reinforcement of the fish immune system. On the present study four isoenergetic and isonitrogenous diets with 10% FM and 6% FO levels supplemented with 5000 ppm galactomannanoligosaccharides (GMOS), 200 ppm of a mixture of essential oils (PHYTO) and a combination of both products, 5000 ppm galactomannanoligosaccharides plus 200 ppm of a mixture of essential oils (GMOSPHYTO). Fish were fed the experimental diets in triplicate for 9 weeks and then fish were subjected to a stress confinment (S treatment) challenge combined or not with an experimental intestinal infection with *Vibrio anguillarum* (SI treatment). Along the challenge test, selected stress and immunological parameters were evaluated at 2h, 24h and 7 days post S or SI treatment. As stress indicators, plasmatic cortisol and glucose levels as well as gene expression of *cyp11β-hydroxylase, hypoxia.inducible factor, steroidogenic acute regulatory protein, heat shock protein 70 and heat shock protein 90* (CYP11β, HIF, StAR, HSP70 and HSP90) were measured. As immune response markers, serum and skin mucus lysozime levels, bactericidal and peroxidase activities as well as gen expression of *Caspase -3* (Casp 3) and *interleukin 1β* (IL-1β) were measured. Besides, fish survival rate to *V. anguillarum* was monitored at the end of the challenge test. Fish fed GMOS and PHYTO diets increased fish relative