have previously been identified and we are interested in studying whether TLR1 and TLR2 form heterodimers, and if so, how this affects the ligand-binding ability.

* Corresponding author.
E-mail address: geert.wiegertjes@wur.nl (G.F. Wiegertjes)

P-400.

Early stimulation of the immune system of an important aquaculture fish species: Probiotic application in European sea bass juveniles

A. Franke, O. Roth, C. Clemmesen.*

GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Abstract
The high mortality during fish early life stages is a major bottleneck in aquaculture. Therefore, the establishment of methods to prevent and control diseases, to ensure efficient growth and to reach maximal survival rates is mandatory to optimize the productivity. A promising solution can be the early activation of the immune system by administration of probiotics as nutritional supplements.
In our study we assess the effect of the probiotic candidate *Bacillus subtilis* on the innate and adaptive immune response of juvenile European sea bass (*Dicentrarchus labrax*). Therefore, Artemia nauplii were used as live carriers to feed *B. subtilis* to 3-month-old sea bass over a period of 2 weeks. Subsequently, the juveniles were fed another week without administering *B. subtilis* in order to estimate the bacterial mucus-binding ability. During the course of the experiment, we evaluated direct effects on the cellular immune response by fluorescence-activated cell sorting analysis and on survival. As a next step we will determine profiles of immune gene expression. To estimate cellular stress, the expression level of metabolism- and stress-related genes will be measured. Furthermore, the RNA/DNA ratio as an indicator of growth will be analysed.

* Corresponding author.
E-mail address: afranke@geomar.de (A. Franke)

P-269.

RNA-seq analysis reveals a complex immune response against saxitoxin in the mussel *Mytilus chilensis*

C. Gallardo-Escárate, G. Núñez-Acuña.*

Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, PO Box 160-C, Concepcion, Chile

Abstract
Saxitoxin (STX) is one of the main phycotoxin that contributes to paralytic shellfish poisoning (PSP), and mainly produced by marine microalgae of the genus *Alexandrium*. This toxin affects a wide range of species including natural populations of marine bivalves. Despite its widely studied effects on the bivalve physiology, the knowledge about how the organisms can detect and translate an immune response against marine toxines is yet scarce. To address this point, we performed a RNA-seq analysis in the mussel *Mytilus chilensis* exposed to SXT. Herein, cDNA libraries from hemocytes of mussels exposed to STX were sequenced by a Hi-Seq 2000 platform. Eighteen individuals were injected with a purified stock of STX (80 μL/100 mg meat) and sampled at different exposition times (0, 4, 8, 16, 24 and 48 hours). Eight hundred millions of reads were yielded and assembled into 225,336 contigs, which were compared with a database enriched for invertebrate immune-related genes. A total of 8% of contigs were successfully annotated, including novel STX-interacting transcripts such as transferrins, calmodulins, voltage-dependent sodium, and calcium/potassium channels. Furthermore, cell receptors such as Toll-like receptors (TLR), lectins and peptidoglycan recognition proteins (PGRP) were also identified. RNA-seq analysis evidenced several transcripts overexpressed at 16 h in individuals injected with STX, suggesting a putative immune response against this phycotoxin. Here, candidate TLRs were discovered as putative cell receptors involved in the immune response to SXT. This study is the first transcriptome overview that reveals a complex immune response to marine toxins in the mussel *Mytilus chilensis*.

* Corresponding author.
E-mail address: crisgallardo@udec.cl (C. Gallardo-Escárate)

P-314.

High-throughput transcriptome sequencing for SNPs discovery associated to immune-relevant genes in the mussel *Mytilus chilensis*

G. Nuñez-Acuña, V. Valenzuela-Muñoz, C. Gallardo-Escárate.*

Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, PO Box 160-C, Concepcion, Chile

Abstract
Single Nucleotide Polymorphisms (SNPs) are highly abundant markers, which are evenly distributed throughout the genome, and due to their potential for high genotyping efficiency and automation, they have rapidly become the marker of choice for genome-wide association studies. In addition, the SNPs variants present in coding regions allow the association with resistant or susceptible phenotypes for specific diseases. However, the lack of large numbers of SNPs associated to immune-related genes represents a bottleneck in most aquatic species. This study reports a high-throughput transcriptome sequencing from the mussel *Mytilus chilensis*, and the SNPs discovery associated to immune-relevant genes. Herein, pooled samples of hemocytes immune-stimulated were sequenced in a Hi-seq 2000 Illumina platform. A total of 225,336 contigs were de novo assembled from 800 millions of reads, yielding 25,860 SNPs into 8,270 consensus sequences. Here, SNP frequency of 1/120 base pair was archived. Furthermore, multi-Blast analysis showed 1,974 (~24%) sequences annotated to immune-related genes such as toll-like signaling pathway. Gene cluster analysis associated to high level of base pair polymorphism is discussed.

* Corresponding author.
E-mail address: crisgallardo@udec.cl (G. Nuñez-Acuña)

P-239.

Cloning of common carp (*Cyprinus carpio*) SOCS1-4 genes and modulation of gene expression by GH-transgene

Q. Gao [1,], Y. Ni [1,2], D.-L. Liu [2], P. Nie [1,*].*

[1] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China;
[2] College of Life Science, Central China Normal University, Wuhan 430079, China

Abstract
Previous studies on immunological parameters of "all fish" growth hormone (GH) transgenic carp suggested that GH transgenic fish exhibited not only the acceleration in growth but also the enhancement in serum lysozyme activity and phagocytosis of hemocytes. However, the mechanism involved in the non-specific immune response and metabolic regulation for GH transgenic carp is unknown. The suppressors of cytokine signaling (SOCSs) are induced by cytokines and act as negative regulators to inhibit cytokine signal transduction. In mammals, the members in the SOCS gene family were identified to regulate a lot of important physiological activities and pathological processes through JAK/STAT system. In the present study, we have cloned SOCS-1, SOCS-2, SOCS-3a, SOCS-3b and SOCS-4 genes from the common carp (*Cyprinus carpio*) on the basis of the construction of tissue cDNA library and homological sequence search, and examined the expression of the five SOCS genes in different development phases and tissues in the GH transgenic and age-matched non-transgenetic control common carp, in order to illustrate the effect of GH transgene on the expression pattern of SOCS genes. The full-length cDNA of common carp SOCS-1/-2/-3a/-3b/-4 is 1237 bp, 1380 bp, 1172 bp, 2216 bp and 1246 bp,

respectively. Open reading frame (ORF) of 606 bp, 594 bp, 630 bp, 660 bp and 1140 bp, codes for the protein of 201 aa, 197 aa, 209 aa, 219 aa, and 379 aa, respectively. Carp SOCS1-4 molecules are well conserved especially in the Src homology 2 (SH2) domain and the extended SH2 domain (ESS), and the SOCS box with B/C box and Cullin box motifs. In addition, Carp SOCS-1/-3a/-3b is conserved in the kinase inhibitory region (KIR). Their tissue expression pattern and dynastic during embryogenesis and fry growth are in process of analysing. Our further aim is to elucidate the function of fish SOCSs and the molecular mechanism for the improvement of the non-specific immune function in GH transgenic carp.

* Corresponding authors.
E-mail addresses: gaoqian@ihb.ac.cn (Q. Gao), pinnie@ihb.ac.cn (P. Nie)

P-287.

Isolation of resident mast cells from sea bream (*S. aurata*) peritoneal exudate

E. García-García[*], *N.E. Gómez-González, A. García-Ayala, V. Mulero*[*].

Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, Campus Universitario de Espinardo, 30100 Murcia, Spain

Abstract
Inflammation is the first response of animals to infection or tissue damage. Histamine has a prominent role in the regulation of inflammation, in vertebrates organisms capable of storing it in specialized immune cells, named mast cells. The perciform *Sparus aurata* was the first fish species shown to possess histamine-containing mast cells at mucosal tissues. It was later found that, similarly to the rat model, the peritoneal exudate of *S. aurata* was enriched in mast cells. Considering the prominent role of mast cells as key regulators of inflammation in vertebrates, a comparative study of the functions of these cells will shed light into the evolution of the cellular and molecular mechanisms involved in the activation of the inflammatory process. We have devised a separation protocol for obtaining highly enriched (over 95% purity) preparations of resident mast cells. The peritoneal exudate of *S. aurata* is composed of lymphocytes, acidophilic granulocytes, macrophages and mast cells. We separated the lymphocyte fraction through density gradient centrifugation on Ficoll-Histopaque. The remaining cell types were cultivated overnight in RPMI-1640 culture medium containing 5% fetal calf serum, which allowed macrophages to adhere to the culture flasks. Finally, granulocytes were separated from the mast cells though a Magnetic-Activated Cell Separation (MACS) protocol, using a monoclonal antibody against these cells. The purity of these enriched mast cells fractions was analyzed by flow cytometry and transmission electron microscopy. This separation protocol will serve as a stepping stone for *in vitro* and *in vivo* studies addressing the evolution of vertebrate inflammatory mechanisms.

* Corresponding authors.
E-mail addresses: erick.garcia@um.es (E. García-García), vmulero@um.es (V. Mulero)

P-476.

Orchestrated interaction between IgT and complement C3 to control a skin parasite of rainbow trout

D. Gómez[1], *Z. Xu*[1], *L. von Gersdorff Jørgensen*[2], *K. Buchmann*[2], *J.O. Sunyer*[1,*].

[1] Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA;
[2] Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Abstract
The complement system is important for the clearance of pathogens from the organism. Via the classical pathway, the complement system is activated by antigen-antibody. In previous analysis of skin mucosal responses of rainbow trout to *Ichthyophtirius multifiliis* (Ich) we discovered that IgT was the main immunoglobulin involved in the response against this pathogen, and that Ich theronts allocated in the skin of infected fish were strongly covered with IgT. With the purpose to elucidate a mechanism by which IgT could be controlling Ich infections, we studied the possible involvement of the complement system. By western blotting we showed that skin mucus contained significant levels of the trout complement protein C3-1. Using immunohistochemistry we discovered that Ich trophonts allocated in the skin of infected fish after 3 weeks post-infection were strongly covered by C3-1 in addition to being coated by IgT. Furthermore, specific tests on skin mucus allowed us to discover that only IgT, but not IgM, could activate C3-1. All these results suggest that the complement may have an important role, together with IgT, in the elimination of pathogens in mucosal surfaces.

* Corresponding author.
E-mail address: sunyer@vet.upenn.edu (J.O. Sunyer)

P-300.

A peptide derived from the D1 domain of *Vibrio anguillarum* flagellin modulates cytokine expression in gilthead seabream and rainbow trout

R. González-Stegmaier[1], *F. Guzmán*[1,2], *A. Romero*[3], *V. Mulero*[4], *L. Mercado*[1,2,3,4,*].

[1] Laboratorio de Genética e Inmunología Molecular, Grupo Marcadores Inmunológicos, Pontificia Universidad Católica de Valparaíso, Chile;
[2] NBC, Núcleo de Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile;
[3] Laboratory of Biotechnology and Aquatic Pathology, Faculty of Veterinary Science, Universidad Austral, Campus Isla Teja, Valdivia, Chile;
[4] Departament of Cell Biology and Histology, Faculty of Biology, University of Murcia, Murcia, Spain

Abstract
Flagellin, the major component of the flagellum in Gram positive and Gran negative bacteria, is described as a ligand of Toll-like receptor 5 (TLR5). In rainbow trout, a soluble form of TLR5 (TLR5S) which is not present in mammals has been described. Flagellin induces the activation of membrane-bound TLR5 (TLR5M), facilitating the production of TLR5S, which apparently amplifies the cellular response mediated TLR5M, allowing a greated immune response. It also induces the expression of proinflammatory cytokines and chemokines, as well as the upregulation of co-stimulatory molecules on dendritic cells, which are essential for T cell activation. In this work we designed and synthesized a peptide analogous to domain D1 of flagellin of Vibrio anguillarum, to evaluate its potential use as an immunomodulator in fish. Peptide design was based on the D1 domain, a conserved region in flagellin molecules that interacts with TLR5S and TLR5M receptors. The peptide was chemically synthesized using F-moc strategy and its purity and molecular weight was confirmed by HPLC and mass spectroscopy, respectively. To evaluate the effect of the synthetic peptide, TLR5M activation through TLR5S expression in cultured rainbow trout liver cells and the expression of the cytokine IL-1β and the chemokine IL-8 on seabream and rainbow trout macrophages was tested. Transcript expression analyses by qPCR showed that the peptide was able to activate TLR5M, stimulating at 6h post stimulus the expression of TLR5S in hepatocytes. Additionally, the peptide increased the expression of IL-1β and IL-8 at 3h post stimulus in rainbow trout and seabream macrophages, with an increase of over 100 times for both transcripts in seabream and 2.5-fold for IL-8 in rainbow trout. The peptide produces a significant immune response in both species in vitro, suggesting its potential use as immunomodulator in farmed fish.

* Corresponding author.
E-mail address: lmercado@ucv.cl (L. Mercado)