CD3 chains play key roles in the development and activation of T cells in higher vertebrates. In the present study, a complete cDNA sequence of CD3ε chain was identified from a sea bass gills transcriptome. Realtime PCR was employed to investigate the basal quantitative levels of CD3ε in tissues and lymphoid organs of sea bass juveniles, with the highest expression found in the thymus followed by gut, spleen, head kidney (HK), peripheral blood leukocytes (PBL) and gills. In vitro stimulation of HK leukocytes with either T-cell mitogen PHA or sea bass recombinant IL-2, resulted in a significant increase of CD3ε transcripts compared to control cultures. The CD3ε cytoplasmic tail region was also identified and used to select three peptides as immunogens in rabbits, in order to produce a polyclonal antiserum. The antibody, named Ra CD3ε1, recognized the immunization peptides in ELISA and stained a band of the expected size in WB at ca. 17 kDa. The distribution and number of CD3ε+ lymphocyte population in the lymphoid organs, mucosal tissues and PBL were addressed in healthy fish by IHC, IIF and flow cytometry, with relatively high percentages of these cells detected among thymocytes, HK, gill and gut leukocytes and PBL, while moderate percentage were found in splenocytes. At the microscope the IIF-positive cells had the typical lymphocyte morphology and a subset of uniquely stained CD3ε+ IgM- cells fit the expected T cell profile. Oral stimulation with a Vibrio anguillarum vaccine increased the CD3ε expression level in HK leukocytes, suggesting that T CD3ε+ lymphocytes may play important roles in the systemic protection against pathogens. Finally, the in vivo modulation of CD3ε+ T intestinal lymphocytes was investigated in fish fed on diets where 50% or 100% fish meal was replaced with the marine-water microalgae Nannochloropsis sp. biomass. IHC revealed a significant enhanced density of T CD3ε+cells in the mucosa of mid intestine compared to fish fed on a control diet. These data suggest that CD3ε+ T lymphocytes may be involved in dietary intestinal immune responses.

Research partially funded by the project ALGAFISH (PTDC/MAR-BIO/6233/2014).

Keywords: CD3εsea bass, polyclonal antibody, immune responses, T cells

Corresponding author.
E-mail address: picchietti@unitus.it (S. Picchietti).

O-085.

Identification and characterization of plasma-like cells in grass carp

Z.-W. Cui [1], X.-Y. Zhang [1], X.-J. Zhang [2], Y.-A. Zhang [1,2,#].

[1] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China
[2] College of Fisheries, Huazhong Agricultural University, Wuhan, China

Abstract
In mammals, plasma cells are well characterized, and CD40L and IL-21 can induce plasma cell differentiation and immunoglobulin production. In this study, we developed monoclonal antibody against grass carp IgM and identified two different IgM+ B cell subsets, namely small IgM+ B cells and large IgM+ B cells. The large IgM+ B cells were further identified as plasma-like cells because they showed gene expression patterns similar with those of human plasma cells and a great capacity to secrete IgM. The small and large IgM+ B cells from either healthy grass carp or the fish stimulated with LPS or Poly (I:C) showed similar phagocytic activity. Recombinant CD40L or IL-21 alone could induce plasma-like cell generation and IgM secretion. Compared with CD40L or IL-21 alone, the combination of CD40L and IL-21 had greater effect on IgM secretion, but not on plasma-like cell generation. These results suggest that plasma-like cells in teleost fish have their own features, such as expressing cell surface IgM and possessing phagocytic activity.

Keywords: B cell, plasma-like cell, CD40L, IL-21, grass carp

Corresponding author.
E-mail address: yonganzhang@mail.hzau.edu.cn (Y.-A. Zhang).

O-086.

Formulation of new adjuvants to be used in fish vaccines

F. Fontenla-Iglesias [1], I. Folgueira [2], A. Riaza [3], J. Lamas [1], J. Leiro [2,#].

[1] Biología Celular, Dpto. de Biología Funcional, Facultad de Biología e Instituto de Acuicultura, Universidad de Santiago de Compostela, Santiago de Compostela, Spain
[2] Dpto. de Microbiología y Parasitología, I. de Investigación y Análisis Alimentarios, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain
[3] Stolt Sea Farm, C/Letonia,Nº2. Edif. Quercus IP;B-2, 15707 Santiago de Compostela, Spain

Abstract
Vaccination is considered the best way of controlling infectious diseases in aquaculture. However, most of the adjuvants used in aquaculture, especially oil-based adjuvants, cause damage to fish, with consequent retardation of growth and negative effects on the welfare of the fish. In this study we developed and tested several adjuvants formulated with carboxymethylcellulose, ulvan or chitosan as the main components. We prepared and tested five formulations, including carboxymethylcellulose-methacrylate gel, ulvan-methacrylate gel, ulvan gel, ulvan-chitosan gel and chitosan gel. All formulations were tested in turbot (*Scophthalmus maximus*) and were administered alone or in combination with particulate antigen obtained from the ciliate parasite *Philasterides dicentrarchi*. Control groups of fish were injected with PBS, antigen or a vaccine containing an oil-based adjuvant (positive group). Fish were injected i.p. on days 0 and 30 with the appropriate vaccine formulation, and on day 60 the fish were examined for intraperitoneal lesions and to determine growth and specific serum immunoglobulin levels (IgM). None of the formulations caused important internal lesions (only small adhesions between internal organs and the peritoneal wall at the injection site), and they did not affect fish growth. Among all the formulations, the chitosan gel yielded the best response in terms of fish serum antibody levels. We also analyzed the immune response generated by the formulations in CD1® IGS mice. Mice were injected i.p. with FCA, aluminum hydroxide, carboxymethylcellulose-methacrylate gel, ulvan-methacrylate gel, ulvan gel, ulvan-chitosan gel or chitosan gel and *P. dicentrarchi* antigen. The best responses in terms of serum antibody levels were obtained with ulvan-methacrylate gel and chitosan gel. The values obtained were similar to those obtained in response to FCA and significantly lower than those obtained in response aluminum hydroxide. In addition, we compared the polarization of th1/th2 response in mice injected with these adjuvants. The results obtained suggest that alternative adjuvants, which induce a good immune response and do not cause important internal lesions, can be formulated for inclusion in fish vaccines.

ACKNOWLEDGEMENTS: This work was funded by EU H2020 program through ParaFishControl Project (634429), by the Ministerio de Economía y Competitividad (Spain) and FEDER (European Union) (AGL2017-83577-R) and by grant ED431C2017/31 from the Xunta de Galicia. F.F. was contracted by the Xunta de Galicia and I.F. was contracted via the M.E.C project.

Keywords: Turbot, vaccine, *Philasterides dicentrarchi*, ulvan, chitosan, immune response

Corresponding author.
E-mail address: josemanuel.leiro@usc.es (J. Leiro).

O-087.

Formulation of *A. salmonicida* adjuvanted vaccine for rainbow trout : Impact of the adjuvant oil origin

K. Veenstra [a,b,#], G. Ionkoff [b], H. Imbault [b], N. Versillé [b], J. Ben Arous [b], C.J. Secombes [a].

[a] Scottish Fish Immunology Research Centre, Institute of Biological and Environmental Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB24 2TZ, UK
[b] SEPPIC SA, Paris La Défense - 50 boulevard National, CS 90020 - 92257, La Garenne Colombes Cedex, France

Abstract
Injectable fish vaccines are mainly based on inactivated antigens which require an adjuvant to trigger a strong immune response. Water-in-oil emulsified vaccines are currently used in the aquaculture industry due to their cost effectiveness, stability and ability to confer long term immunity. However, oil-adjuvanted vaccines can be reactogenic and induce side effects in fish. In this study, we analyzed the impact of the oil origin of the adjuvant on *Aeromonas salmonicida* vaccines safety and immunogenicity. Two different adjuvants were tested, one based on a non-mineral oil (Montanide™ ISA 763A VG) and one based on a mineral oil (Montanide™ ISA 761 VG). Following intraperitoneal vaccination of rainbow trout, blood samples were taken at 42 and 53 days post vaccination (dpv) to assess antibody response, and adipose tissue samples were collected at 3, 14 and 28 dpv for RT-qPCR analysis of immune genes implied in the pro-inflammatory and adaptive responses. Side effects in the peritoneum were scored at 7, 14, 28, 42 and 53 dpv. Both vaccines induced a high antibody response against A. salmonicida with a significant increase in titre between 42 dpv and 53dpv. Vaccination-induced adhesion scores for the vaccine groups fell within industry-accepted limits as per Spielberg Standardized Extended Post-Vaccine Scoring. However scores were lower for the fish vaccinated with the non-mineral oil adjuvant. Compared to the control group (antigen alone), a clear upregulation of immune genes occurred in response to both vaccine groups, which persisted over time. This upregulation was higher for fish vaccinated with the mineral oil adjuvant. Furthermore, a strong correlation between gene expression, modulated by the oil origin, and vaccine safety was observed. These results showed that oil origin of fish adjuvants has an important impact on the immunogenicity and safety profile of fish vaccines, and that Montanide™ ISA 763A VG and Montanide™ ISA 761 VG are efficient adjuvants for the formulation of inactivated *A. salmonicida* vaccines.
Keywords: Inactivated vaccine, fish, oil, adjuvant, safety

Corresponding author.
E-mail address: kimberly.veenstra@fli.de (K. Veenstra).

O-088.

Long-term, proteome-scale analysis of rainbow trout immune proteins: Implications for aquaculture vaccine development

F.K. Bakke [1], M.M. Monte [2], D. Causey [1], T. Cornulier [1], A. Douglas [1], D. Stead [3], S.A.M. Martin [1], D.J. Macqueen [4,#], H. Dooley [5,#].

[1] School of Biological Sciences, University of Aberdeen, UK
[2] Laboratory of Immunology-Vaccinology, Faculty of Veterinary Medicine, University of Liège, Belgium
[3] Aberdeen Proteomics, The Rowett Institute, University of Aberdeen, Aberdeen, UK
[4] The Roslin Institute and Royal (Dick) Veterinary School, University of Edinburgh, UK
[5] University of Maryland School of Medicine, Baltimore, MD, USA

Abstract
Infectious diseases pose a significant threat to the economic stability and expansion of finfish aquaculture. Vaccination is widely considered the best prevention strategy, but evaluation of immune protection typically relies on measuring immune gene expression at the mRNA level from terminally-acquired tissue samples. However, mRNA expression does not always correlate with tissue protein levels, providing an incomplete representation of the nature and kinetics of the immune response. In addition, inter-individual variation necessitates the use of large numbers of experimental animals to obtain sufficient statistical power. To overcome these limitations, we used a long-term, proteome-scale approach to identify and quantify changes in immune protein levels in rainbow trout (*Oncorhynchus mykiss*) plasma. These changes provide an indication of fish health and immune status, while also permitting non-lethal sampling. Although all experimental fish mounted an antigen-specific humoral response, the timing and magnitude of this, and the response trajectories of most immune-relevant proteins, differed markedly between individuals. However, certain immunological proteins were found to be more consistently expressed across all fish, and may represent useful biomarkers of the immune response. Together our data emphasise the importance both of judicious selection of immunological biomarkers, and of careful assessment of changes in the expression of such proteins over longer-term study periods, when considering whether or not an effective antigen-specific immune response has been mounted. More generally, this approach offers a useful tool to monitor fish immune responses, while dramatically reducing the number of experimental animals required.
Keywords: Proteomics, vaccination, non-lethal sampling, individual variation, biomarkers.

Corresponding authors.
E-mail addresses: daniel.macqueen@roslin.ed.ac.uk (D.J. Macqueen), hdooley@som.umaryland.edu (H. Dooley).

O-089.

A live attenuated strain of HY9901ΔvscB provides protection against *Vibrio alginolyticus* in orange spotted grouper (*Epinephelus coioides*) model

H. Pang [1,2,3,], Y. Chang [1,2,3,*], P. Wu [1,2,3,*], S. Zhou [1,2,3], R. Hoare [4], S. Monaghan [4], D. Song [1,2,3], Y. Ding [1,2,3,#], J. Jian [1,2,3,#].*

[1] College of Fishery, Guangdong Ocean University, Zhanjiang 524025, China
[2] *Guangdong Provincial Key Laboratory of Pathogenic Biology and Epidemiology for Aquatic Economic Animals*, Zhanjiang, 524025, China
[3] *Guangdong Key* Laboratory of Control for Diseases of Aquatic Economic Animals, Zhanjiang 524025, China
[4] Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, Scotland, UK

Abstract
Vibrio alginolyticus, a bacterial pathogen in fish and humans, expresses a type III secretion system (T3SS) that is critical for pathogen virulence and disease development. In this study, the T3SS gene vscB was cloned from *V. alginolyticus* wild-type strain HY9901 and the mutant strain HY9901&vscB was constructed by the in-frame deletion method. The HY9901&vscB mutant showed an attenuated swarming phenotype and a 23-fold decrease in the virulence to grouper. However, the HY9901&vscB mutant showed no difference in morphology, growth, biofilm formation and ECPase activity. Finally, grouper vaccinated via intraperitoneal (IP) injection with HY9901&vscB induced a high antibody titer with a relative percent survival (RPS) value of 77.6% after challenging with the wild-type HY9901.Real-time PCR assays showed that vaccination with HY9901&vscB enhanced the expression of immune-related genes, including MHC-I3, MHC-II3, IgM, IL-1β, TNF-3 and CD83 after vaccination, indicating that it is able to induce humoral and cell-mediated immune response in grouper. These results demonstrate that the HY9901&vscB mutant could be used as an effective live vaccine to combat *V. alginolyticus* in grouper.
Keywords: *Vibrio alginolyticus*; T3SS; vscB; live attenuated vaccine; *Epinephelus coioides*

Corresponding author.
E-mail addresses: dinv@foxmail.com (Y. Ding), jianjic@gmail.com (J. Jian).
* These author shave contributed equally to this work.