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O-001

EFFECT OF A PHYTOGENIC FEED ADDITIVE ON THE GROWTH PERFORMANCE AND IMMUNITY OF PACIFIC WHITE LEG SHRIMP, *Litopenaeus vannamei*, FED A LOW FISHMEAL DIET

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ABSTRACT
Fishmeal has long been one of the most important ingredients in formulated aquafeeds, due to its high protein content, excellent composition of essential amino acids, and high digestibility. Increasing economic and ecological concerns regarding the use of fishmeal have encouraged the development of replacement strategies. Plant-derived products have been suggested to partially replace fishmeal in aquafeeds, however often at decreased growth performance, inflammatory responses, and increased susceptibility to diseases. This study assessed the effects of the commercial phytogenic feed additive Digestarom® PEP MGE on the growth, nutritional performance, and immune response of *Litopenaeus vannamei*. Juvenile shrimp (N=540) were stocked in 36 tanks (V = 100 L) for 63 days and fed one of the four experimental diets: i) standard formulation (control, 24% fishmeal), ii) low fishmeal diet (5%), iii) low fishmeal diet plus 0.2 g/kg Digestarom® PEP MGE, and iv) low fishmeal diet plus 0.4 g/kg Digestarom® PEP MGE. The results obtained after 63 days of feed supplementation suggest that the blend of essential oils tested compensated for the negative performance and health consequences of the low fishmeal diet. Particularly, the survival, FCR, total hemocyte count, and respiratory burst of the shrimp fed a low fishmeal diet supplemented with this phytogenic improved up to the levels recorded for shrimp fed a high fishmeal diet. Overall, results suggest that Digestarom® PEP MGE can be incorporated into shrimp low fishmeal diets to compensate for the negative performance and immunological effects of partially replacing fishmeal with plant-based protein.

KEYWORDS
phytogenics, low fishmeal, white leg shrimp, growth performance, immunity
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O-002

MEMBRANE ASSOCIATED PROTEIN FLOTILLIN IN *Litopenaeus vannamei* PLAYS A ROLE IN WSSV INFECTION

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Abstract

Flotillin, an important protein of vesicular endocytosis, plays an essential role in a large number of cellular processes, including viruses and pathogen infection. In the present study, a *flotillin-2* homolog in *Litopenaeus vannamei*, designed as *Lvflotillin-2*, was cloned and characterized. To analyze the putative role of Lvflotillin-2 during white spot syndrome virus (WSSV) infection, real-time quantitative PCR was performed. The result showed that the transcriptional level of *Lvflotillin-2* was up-regulated significantly after virus challenge. Furthermore, upon WSSV stimulation, Lvflotillin-2 in shrimp cells could translocate from the plasma membrane to intracellular compartments, and unexpectedly, also into nucleus. Additionally, depletion of *Lvflotillin-2* inhibited WSSV gene *ie1* transcription. These observations indicated that Lvflotillin-2 was involved in viral infection and WSSV stimulation resulted in its dynamic localization.

KEYWORDS

Shrimp, flotillin-2, WSSV, endocytosis

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O-003

COMPLEXITY OF *Penaeus monodon* DSCAM GENE STRUCTURE OCCURS IN BOTH EXTRACELLULAR REGION AND CYTOPLASMIC TAIL

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ABSTRACT
In pancrustaceans, the Down syndrome cell adhesion molecule (Dscam) is an extraordinarily labile gene; thousands of isoforms can be generated by combining alternatively spliced exons from a single-locus gene. In insects, Dscam is involved in immunity as a hypervariable immune receptor. Similarly, we reported that Dscam was a hypervariable immune receptor in shrimp and crayfish. Interestingly, a unique tail-less Dscam identified in shrimp lacked a transmembrane domain and cytoplasmic tail. However, the mechanism to produce this unique tail-less Dscam is unknown. Here, we determined that the *P. monodon* Dscam (*Pm*Dscam) genome was ~250 kbp. The extracellular region had 10 immunoglobulin domains and six fibronectin III domains, i.e., [Ig1-Ig9]-[FNIII 1-FNIII 4]-[Ig10]-[FNIII 5-FNIII 6], with half of the second and third Ig domains and the entire Ig7 domain encoded by exon duplication. There were 26, 81, and 26 alternatively spliced exons in the Ig2, Ig3, and Ig7 domains, respectively, with potential to generate >54,000 protein isomorphs in the extracellular region of *Pm*Dscam. A very complex cytoplasmic tail structure was retrieved from this gene organization analysis. We identified three stop codon sites on the single gene sequence; furthermore, several exons encoded for cytoplasmic tail have also been identified. Taken together, *Pm*Dscam has potential to generate >21,000,000 unique isoforms via alternative splicing of both extracellular region and cytoplasmic tail, the highest potential number of isoforms among those crustaceans. In conclusion, we inferred that shrimp Dscam can use an alternative splicing event to produce selective isoforms against pathogens. Furthermore, Dscam may mediate specific immune responses in shrimp.

KEYWORDS
Dscam, gene structure, genome, immunoglobulin family, shrimp
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O-004

ARTEMIA BIOENCAPSULATION DELIVERS SULFATED GALACTANS TO TISSUES AND ACTIVATES THE EXPRESSION OF IMMUNE GENES IN SHRIMP

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ABSTRACT
Live food has been used for feeding and delivery of the compounds to larval stages of aquatic animals. *Artemia* can be used as a possible vector for the delivery of different substances such as nutrients, probiotics and immune-stimulants. Sulfated galactans (SG) from *Gracilaria fisheri* has been demonstrated to stimulate immune activity and against pathogenic infection in shrimp. In vitro study revealed that SG bound with shrimp haemocyte and activated the expression of immune related genes in haemocyte culture. The present study was carried out to investigate the bioencapsulation of *Artemia* with SG for delivery to tissues and activating the expression of immune genes in shrimp. SG conjugated with fluorescein isothiocyanate (FITC-SG) showed no significant toxic in *Artemia*. Bioencapsulation of *Artemia* with FITC-SG (0-100 µg/ml) revealed that FITC-SG was located in *Artemia* gut lumen with a time and dose dependent manners. Tracking of SG in shrimp, shrimp fed with *Artemia* bioencapsulated with FITC-SG demonstrated that FITC-SG presented in shrimp gut until 2 h after feeding. Twenty-four hours after feeding, shrimp tissues were collected and the distribution of FITC-SG was observed in gill, hepatopancreas and haemolymph. FITC-SG was also found to bind with shrimp haemocyte. In addition, shrimp continuously fed with *Artemia* bioencapsulated with SG for 3 days showed activated the expression of immune genes including IMD, IKK□, dicer and proPO-I, and prolonged high expression levels for a week. These results indicated that *Artemia* bioencapsulation could deliver the SG passed through gut to tissues and bound with haemocyte, and subsequently activated expression of immune genes in shrimp.

KEYWORDS
Artemia bioencapsulation; Sulfated galactans; FITC-SG; Shrimp haemocyte; Immunity
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O-005

VACCINATION STRATEGIES AND IGM RESPONSES AGAINST PKD IN RAINBOW TROUT

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ABSTRACT
PKD is one of the most serious diseases affecting trout aquaculture in the UK. Caused by the myxozoan parasite, *Tetracapsuloides bryosalmonae*, PKD is elicited by the temperature-dependent development of parasite spore sacs in colonial bryozoans. Since recovered fish are known to exhibit protective immunity to re-infection, a successful treatment, based on the reduction of kidney pathology and parasite burden, could markedly reduce fish mortalities leading to improved productivity and fish welfare. Our investigations have focused on the selection of putative *T. bryosalmonae* virulence factors to unravel host immune evasion mechanisms exploited by the parasite, whilst shortlisting candidates for vaccine studies.
Here we report the results of three DNA-vaccination field trials using selected antigens administered individually or in combination. Some vaccine groups were found to have a partial protective effect in reducing PKD-associated kidney pathology whilst decreasing parasite load**.** Pathology reduction was improved in successive trials by improving the vaccination strategy.
We have functionally characterized the most promising antigen from our vaccine studies, which represents a novel micro-exon gene (*Tb*-MEG1). MEGs, until now, were thought to be unique to helminth parasites. In schistosomes, they exhibit extensive antigenic variability that is thought to enable greater plasticity in host protein targeting as a mechanism of host immune subversion over the course of infection. Using a validated anti *Tb*-MEG1 MoAb, we have demonstrated the protein to be expressed in and on the surface of parasites and a subset of immune cells within the kidneys of infected fish. We have also demonstrated potent *Tb*-MEG1-specific IgM responses in sera from parasite-infected (farmed) rainbow trout and have successfully induced a specific IgM response after protein vaccination.
DNA vaccines encoding molecules homologous to proteins involved in nutrition acquisition, cell-cell interactions or of unknown function, have shown promise towards the development of a future PKD vaccine, that may also be applicable to the generation of vaccines against other fish parasites. The discovery and characterization of *T. bryosalmonae* antigens has provided valuable insights into host immune evasion by myxozoan parasites, with the present *Tb*-MEG1 studies also having major implications towards understanding the evolution of antigenic variability in metazoan parasites.

KEYWORDS
Vaccination, myxozoan, parasite, antigen, igm response
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O-006

DEMONSTRATION OF HERD IMMUNITY EFFECTS IN DNA VACCINATED RAINBOW TROUT.

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ABSTRACT
While DNA vaccination by intramuscular injection is known to provide highly protective immunity against viral haemorrhagic septicaemia (VHS) in rainbow trout at an individual level, we here aimed at examination of heard immunity effects. Induction of clinical disease following experimental challenge by waterborne exposure of the fish to the pathogens represents a hurdle in vaccine potency testing for many pathogens, but in the case of VHS virus, challenge can be done efficiently by immersion. However, progression and level of mortality depends on the challenge dose. This has some implications for the experimental design and interpretation of the results. Initial experiments indicated that while mortality following a high challenge dose was independent on whether the fish were kept together in one aquarium or in individual aquaria, indirect infection by virus released from primary infected fish seemed to play an important role at low challenge doses. In experimental vaccination trials, it may be assumed that challenge of cohabitant naïve and vaccinated fish represents a harder test of vaccine potency compared to challenging the vaccinated fish separately due to secretion of high amounts of virus from the naïve fish. In challenge trials with high inoculum of virus, this seemed to be true for VHSV challenge of DNA vaccinated rainbow trout as illustrated by increased mortality among vaccinated fish cohabitated with naïve fish. However, at lower inoculum, the effect tended to be opposite in the sense that mortality among the naïve fish was reduced. Implications of the results for experimental vaccine testing will be discussed.

KEYWORDS Fish rhabdovirus, DNA vaccine, challenge setup, vaccine testing, herd immunity.

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O-007

EXPOSURE TO ANTIBIOTICS AFFECTS SAPONIN IMMERSION INDUCED IMMUNE STIMULATION AND SHIFT IN MICROBIAL COMPOSITION IN ZEBRAFISH LARVAE

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ABSTRACT
In the last decades, pollution of the environment by large scale use of antibiotics in agriculture and human medicine have led to increased antimicrobial resistance in both the environment and the host animal microbiome. Disturbances in the host microbiome can result in impaired immunity and reduced resilience of aquaculture species. Here, we investigated whether environmentally measured levels of the commonly used antibiotics ciprofloxacin and oxytetracycline influences the host microbiome and susceptibility toward saponin-induced immune stimulation in larval zebrafish. Firstly, neutrophil and macrophage reporter zebrafish larvae were exposed to different concentrations of soy saponin by immersion. A dose-dependent increase in neutrophil presence in the intestinal area was observed together with increased expression of immune genes il1b, tnfa, il22 and mmp9. To investigate the effect of antibiotics, larval zebrafish were immersed in ciprofloxacin or oxytetracycline in the presence or absence of a low dose of saponin. In vivo imaging revealed that antibiotic treatment did not reduce the number of neutrophils that were recruited to the intestinal area upon saponin exposure, although it did tend to lower pro-inflammatory cytokine levels. Microbial sequencing of whole larvae revealed that exposure to a low dose of saponin already shifted the microbial composition. The combination of oxytetracycline and saponin significantly increased α-diversity compared to the controls. In conclusion, the current study provides evidence that the combination of low levels of antibiotics with low levels of anti-nutritional factors (saponin) can induce inflammatory phenotypes and can modify the microbiota, which might lead to altered disease susceptibility.

KEYWORDS
Zebrafish, microbiota, saponin, neutrophils, macrophages, antibiotics

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O-008

CAN PASSIVE IMMUNIZATION PREVENT DISEASE OUTCOME IN GILTHEAD SEA BREAM EXPOSED TO *Enteromyxum leei*?

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ABSTRACT
Passive immunization is used in humans for treating or preventing some infectious diseases, but it also constitutes an emerging field of interest in aquaculture, particularly with the restrictions for antibiotic use. Intracoelomically-injected antibodies can be detected in fish sera within the first 8 h and their half-life ranges from 7 to 22 days post-injection, depending on the species. The fish models studied so far used fast-acting pathogens such as virus, bacteria or ciliate parasites. The current work aimed to determine if passive immunization could help to prevent enteromyxosis in gilthead sea bream (GSB, *Sparus aurata*). *Enteromyxum leei* is a myxozoan intestinal parasite that invades the paracellular space of the intestinal epithelium, producing a slow-progressing disease, leading to anorexia, cachexia and mortalities. We have previously demonstrated that GSB that survive *E. leei* infection become resistant upon re-exposure, and this resistance is directly related to the presence of high levels of specific serum antibodies.
In the current study, we evaluated whether injection with sera from resistant animals would protect naïve fish when challenged by effluent exposure to the parasite. Serum from a pool of resistant (R) and naïve (N) animals (intact or heat inactivated, 10 µl/g BW) was intracoelomically injected 24 h prior to the *E. leei*-effluent challenge and at 9 days post-challenge (dpc). At 23 dpc, the different groups were allocated in separate tanks and the effluent exposure was terminated. A non-lethal parasite diagnosis was performed at 56 dpc. At the final sampling (100 dpc), blood, serum and tissues were collected for hematology, circulating antibodies, histological and molecular diagnosis and gene expression.
Groups injected with R sera had lower prevalence and intensity of infection than those with N sera, both in the intermediate and final samplings. At 100 dpc, the prevalence of infection in the PBS and N groups was 70%, whereas in R group it only reached 55%. Condition factor (CF) and specific growth rate (SGR), key parameters affected by enteromyxosis, were higher in R group. There was a significant correlation between prevalence of infection and SGR and CF. Immunohistochemistry and gene expression studies will reveal whether this partial protection was due to higher presence of specific antibodies or specific cell populations. These results show that, even with this long term disease, passive immunization can confer some degree of protection. The administration of specific antibodies during exposure, probably provided fish with time to activate the specific defenses before the parasite proliferated.
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KEYWORDS
Passive immunization, Myxozoa, antibodies, gilthead sea bream, parasites.

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O-009

BACTERIAL MEMBRANE VESICLES AS VACCINES IN AQUACULTURE

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ABSTRACT
Infections by two Gram-negative facultative intracellular bacterial pathogens, namely, *Piscirickettsia salmonis* and *Francisella noatunensis,* are causing major problems in aquaculture world-wide. *F. noatunensis* sp is one of the main factors hampering the development of fish farming based on Atlantic cod in Norway and is deleterious to tilapia, a farmed fish that is produced over 3.5 mill tons/year. *P. salmonis* infections have been devastating for salmon aquaculture. As of today no effective treatments are available against the diseases. The immunologically inaccessible intracellular location of *Francisella* and *Piscirickettsia* have until now complicated the development of protective measures. This is in stark contrast to the successful development of efficient vaccines that has been made possible against important extracellular bacterial infections in salmon based on whole inactivated bacteria injected with oil adjuvants. It has been shown that both *P. salmonis* and *F. noatunensis* secrete membrane vesicles (MV). Bacterial MVs has been shown to contain proteins, DNA and RNA and simulate the mother bacteria in a non-replicative form. Bacterial MV has been reported as potential vaccine candidates for a range of host including humans, mice and fish against infection caused by intracellular pathogenic bacteria as they induce both a humoral and cellular immunity. Here the characterization of MV isolated from *P. salmonis* and *F. noatunensis* is described, and their vaccine potential is verified in a zebrafish infection and vaccine model. The further use of the MVs as vaccines in their natural hosts such as strain-specificity and cross-immunity will be discussed.

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O-010

POTENTIAL ROLE OF RAINBOW TROUT RED BLOOD CELLS AS MEDIATORS IN THE IMMUNE RESPONSE INDUCED BY DNA VACCINES

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ABSTRACT

Fish red blood cells (RBCs), unlike mammals, possess nucleus and organelles in their cytoplasm that give them the necessary machinery to generate an immune response at transcriptional and at proteomic level. In the last years nucleated RBCs have demonstrated to act as phagocytic cells, release cytokine-like factors and modulate leukocyte activity upon different stimulus. Also, they have been implicated in the response against viral infections. And recently, rainbow trout RBCs have been also implicated in the immune response to a DNA vaccine. So far, DNA vaccination is the best strategy to prevent and control viral infections, and for fish rhabdoviruses, only the DNA vaccine based on glycoprotein G (gpG) have resulted effective. However, the whole mechanisms involved in this protection and the immune response triggered by the DNA vaccine remain to be fully understood. In order to investigate the role of nucleated RBCs in DNA vaccination, we evaluated the immune response triggered by a DNA vaccine encoding the gpG of viral hemorrhagic septicaemia virus (VHSV) (GVHSV) in rainbow trout RBCs and explored RBCs as future targets or carriers for DNA vaccination. Upon fish vaccination of rainbow trout with GVHSV DNA vaccine, RBCs upregulated antigen presentation pathways at transcriptome and proteome level. In addition, rainbow trout RBCs responded to the DNA vaccine upregulating interferon type 1 (IFN-1) pathway. Also, RBCs transfected *in vitro* with GVHSV DNA vaccine protected RTG-2 cell line against subsequent viral infection. Besides, RBCs carrying the GVHSV DNA vaccine were able to induce specific antibody against VHSV *in vivo*. Also, RBCs transfected *in vitro* with GVHSV were able to modulate leukocyte activity *in vitro*. In summary, we suggest nucleated RBCs as cell mediators of the immune response playing an active role in DNA vaccination and propose nucleated RBCs as potential cell targets or carriers of antiviral prophylactics.

KEYWORDS
Red blood cells, DNA vaccine, GVHSV, transcriptome, proteome

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O-011

CHARACTERIZATION OF IMMUNE RESPONSES IN DIFFERENT ENVIRONMENTAL TEMPERATURE THROUGH LINEAR ARRAY EPITOPE VACCINE INDUCTION

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ABSTRACT

Grouper fish have a very high value economically and also nutritionally; these make them a very important fish species across the world. However, this fish often suffers a very high mortality rate, up to 90%-100% whenever they are infected with a virus and always bring about a tremendous lost to the farmers of the relative fisheries. As fishes are ectothermic animals, where their bodily temperature and the immune system are regulated by the constant change of environmental temperature, we would like to characterize the vaccine in immune response induction upon virus infection in fish under various modulation of temperatures. Previously, our previous study has developed a vaccine NNVCP-S5E that was developed by a PCR based technique, linear array epitope (LAE) where the immunogen is having multiple linear epitope copies, which were predicted and chosen from NNV and the epitopes are amplified by template-repeat polymerase chain reaction (TR-PCR). Efficacy tests of the vaccine have been done on NNV challenged fishes previously and the relative percent survival (RPS) results were 72%, which indicates that NNVCP-S5E provides an effective prevention against NNV infection. However, the immune response characterized, whether TH1 or TH2 immune pathway is induced by vaccination of LAE vaccine in different temperatures are still unknown. Thus, juvenile giant grouper (*Epinephelus lanceolatus*) is used as a test subject in this project and the fishes were first acclimatized under three temperatures, 20°C, 28°C and 36°C for 1 week, and vaccinated through intraperitoneal injection (IP). Then, the fishes are challenged with purified NNV with a median lethal dosage, LD50 of 3.16.105copies/mL, and sacrificed to obtain immune related organs such. Real-time PCR (RT-PCR) is done in order to investigate the mRNA expressions of the TH1 or TH2 immune pathway markers, *T-bet* for TH1 pathway; *Gata-3* and *c-Maf* for TH2 pathway. Based on the RT-PCR analysis from all three groups, the gene expression results indicate that the vaccination tends to direct the fish immune response towards TH2 pathway of the adaptive immune response, which is a pathway towards the proliferation of B cells and a stronger antibody production. Furthermore, the study also shows a higher survival rate for the fishes that were acclimatized and vaccinated under 35°C compared to other temperatures after challenged with NNV for 14 days. This study contributed different insights which would help in a better protection against virus infection.

KEYWORDS
Linear Array Epitope Vaccine, Grouper, Nervous Necrosis Virus, Temperature Difference, Immune Pathway

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O-012

STRESS HORMONES MODULATE EARLY IMMUNE ACTIVITIES INTHE HEAD KIDNEY OF *Coregonus maraena*

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ABSTRACT

Under challenging conditions including threat and discomfort, the vertebrate stress response triggers endocrine and neurologic networks. They release stress hormones such as cortisol and catecholamines in the head kidney. This endocrine and hematopoietic tissue is thus of central importance for stress effects on the immune response in teleosts. Individual aspects of the interference of stress hormones (mainly cortisol) with immune processes have already been reported in some bony fish. Although less studied, the catecholamines adrenaline and noradrenaline have also shown to modulate the immune response of teleost leukocytes via α and β adrenergic receptors. This study aims to expand the actual knowledge on stress-induced immune modulation, in order to evaluate the effects of stress on the immune system of maraena whitefish (*Coregonus maraena*). This salmonid fish is highly sensitive to stress compared to other salmonid species long adapted to aquaculture. To this end, a large set of specific primers was designed for reverse-transcription quantitative real-time PCR (RT-qPCR) analyses. The primer panel included cell-specific marker genes characterizing the distinct cell populations in the head kidney of *C. maraena*, which had been sorted using flow cytometry. In addition, we analysed the expression of catecholamine and cortisol receptors in each population, in order to define the repertoire of stress-related modulators present in the cells. In the next step, we performed a series of in vitro stimulations of head kidney leukocytes to study the expression of genes involved in immune activation and acute phase together with catecholamine and cortisol receptors. The primary cells were cultured for defined periods of time with adrenaline, noradrenaline or cortisol. In addition, cells were stimulated with highly purified pathogen-associated molecular patterns (PAMPs), either alone or in combination with the above hormones. Our study characterises, on one hand, the cell populations of maraena-whitefish-head kidney and reveals potential stress-response targets. On the other hand, we recorded the impact of stress hormones and PAMPs on the immune activity in head-kidney cells giving insights in the regulatory mechanisms behind the interaction of cortisol and catecholamines with leukocytes during immunological challenges.

KEY WORDS

Cortisol, catecholamines, immune system, gene expression, salmonids.

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O-013

DIVERGENT AND OVERLAPPING FUNCTIONS OF TYPE I INTERFERONS IN ZEBRAFISH

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ABSTRACT

Teleost possess 2 subgroups of type I interferons (IFNs) (group I and II) which bind to distinct receptors to activate antiviral response. Multiple isoforms are common within the subgroups. However, the functional differences of individual IFNs are poorly understood. In zebrafish, IFNphi1 and IFNphil4 belong to the group I IFN subgroup containing 2 conserved cysteines in the mature peptide and share a common heterodimeric receptor consisting of CRFB1 and CRFB5. It has been shown that the IFNphi1 can elicit strong antiviral response and is able to enhance host resistance to viral infection. In contrast, the functions of IFNphi4 are largely unknown. In the present study, we found that the IFNphi1 and IFNphi4 were differentially modulated during bacterial and viral infection. RNA sequencing analyses indicate that the ZF4 cells stimulated with the recombinant IFNphi1 and IFNphi4 proteins showed considerable similarity of expression patterns of genes involved in antiviral responses but also displayed marked differences. The results provide insights into the divergence of type I IFN functions in teleost fish.

KEYWORDS

Interferon, cytokine, function, antiviral response, zebrafish

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O-014

DIRECT CYTOTOXIC ACTIVITY OF CD8+ T CELLS AGAINST *Ichthyophthirius multifiliis* IN GINBUNA CRUCIAN CARP, *Carassius auratus langsdorfii*

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ABSTRACT
A line of studies has shown that several humoral immune factors including complement, lectins and antibodies are involved in protection from parasite infections. However, cell-mediated immunity against parasites has poorly been understood in teleost fish. In the present study, direct cytotoxic activity of leukocytes against *Ichthyophthirius multifiliis* has been demonstrated in ginbuna crucian carp. Leukocytes labeled by each monoclonal antibody (2C3: anti-CD8, 6D1:anti-CD4, GB20: anti-macrophages/neutrophils) were co-incubated with *I. multifiliis*. The fluorescent microscopic observation showed that CD8+ T cells from naïve ginbuna carp, but not other leucocytes, contacted *I. multifilii*. The cytotoxic activity of CD8+ T cells was significantly higher than that of other leucocytes, indicating that CD8+ T cells are dominant effector cells against *I. multifiliis*. The cytotoxic assay using a trans-well insert suggested that CD8+ T cells require to contact the parasites for the direct killing. Furthermore, a serine protease inhibitor 3, 4-dichloroisocoumarin (DCI) inhibited the cytotoxic activity of CD8+ T cells, but a perforin inhibitor Concanamycin A (CMA) did not. These results indicate that teleost CD8+ T cells have natural cell-mediated cytotoxicity against extracellular parasite by utilizing serine proteases, such as granzyme, suggesting that CD8+ T cells play an important role in innate immunity against extracellular protozoan parasites.

KEYWORDS
Ginbuna crucian carp, immune system, CD8+ T cells, cell-mediated cytotoxic activity, *Ichthyophthirius multifiliis*

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O-015

ISOLATION AND CHARACTERIZATION OF SHARK SINGLE DOMAIN ANTIBODIES CAPABLE OF BINDING SALMONID ALPHAVIRUS

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ABSTRACT

Salmonid alphavirus (SAV) causes pancreas disease and sleeping disease in farmed Atlantic salmon and rainbow trout, resulting in significant economic losses to the aquaculture industry. To enable the rapid detection of SAV, robust reagents, capable of providing sensitive and specific detection, are required. Purifying SAV free from cell contaminates is difficult and may explain why there are a lack of commercially available antibodies for SAV. In this study a different approach, utilizing the novel shark immunoglobulin IgNAR, was investigated as a strategy for the production of SAV-detection reagents. IgNAR is a heavy chain homodimer that binds to antigens via a pair of highly soluble, single domains, referred to as VNARs. In this work a recombinant VNAR antibody library was generated from a nurse shark (*Ginglymostoma cirratum*) host immunised with a combination of inactive SAV and recombinant SAV E2 protein. This library was panned using phage display technology to identify SAV-specific VNARs for use as immunological detection reagents. Of the novel VNAR clones identified three bound viable SAV with a high degree of sensitivity. These VNARs were shown to detect SAV subtypes 1, 2, 3, and 5 by ELISA. Two of the VNARs have specificity to SAV E2 glycoprotein. All of the VNARs showed characteristically high resistance to irreversible thermal denaturation. The subtype cross- reactivity and demonstrable robustness of these VNAR domains should enhance their utility as diagnostic reagents in the field.

KEYWORDS
Shark IgNAR; VNAR antibody; Pancreas disease; Salmonid Alphavirus; Phage display
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O-016

INFLUENCE OF HYPOXIA STRESS ON THE IMMUNE RESPONSE OF PIKEPERCH (*Sander lucioperca* L., 1758)

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ABSTRACT
Animal welfare is a main issue in today's aquaculture. Pikeperch (*Sander lucioperca* L., 1758) is a native food fish of the northern hemisphere and very attractive for European aquaculture. It is susceptible to typical stress parameters of ongoing domestication. Unsuitable water temperatures and the lack of oxygen influence the well-being of fish and evoke to stress responses. Oxygen saturations lower than 40% are considered unfavourable for aquaculture facilities. These can be caused by insufficient water circulation, high stocking densities or high water temperatures. Temperatures above 20°C are optimal for pikeperch farming. Above 25°C, the oxygen concentration in water and the growth rate in pikeperch are reduced. These conditions are thus considered almost critical. Acute stress in fish modulates the immune system, including the release of a distinct set of cytokines and acute phase proteins. Chronic stress suppresses the immune system impairing the defense against pathogens. Hypoxia inducible factor (HIF)-1α is the most prominent regulator of hypoxic conditions in vertebrates, including Teleostei. It is expressed in nearly all immune cells and an interesting candidate as possible biomarker for hypoxic stress conditions. Little information has been published on the stress physiology of pikeperch. For successful breeding in aquaculture, details on effects of the stress parameters temperature and hypoxia will be important. We demonstrated recently, that a rise from 15°C to 25°C not only changes the expression pattern of heat shock induced genes (e.g. *HSP90AA1* and *SERPINH1*), but also of the hypoxia induced gene *HMOX1*, in liver and gills. The present study investigates the influence of oxygen deficiency on the early immune response of pikeperch. After peritoneal stimulation with inactivated *Aeromonas hydrophila* cells, we evaluated the transcript levels of possible biomarker genes, cell composition in certain tissues and immune cell activity. Preliminary data show that persistent lymphocytes of the peritoneum decrease in number after stimulation. Myeloid cells invade the site of infection to clear the pathogens. We suggest that hypoxia stress decreases the number of myeloid cells entering the peritoneum and these cells seem to originate from the head kidney.
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KEYWORDS
Pikeperch, hipoxia, welfare, immune System, stress Physiology

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O-017

ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF ETHANOL EXTRACT FROM VERBENACEAE PLANT *Clerodendrum cyrthophyllum* TURZ IN COPPER SULFATE INDUCED INFLAMMATION IN ZEBRAFISH

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ABSTRACT

Oxidative stress and inflammation are commonly present in several chronic diseases. Interestingly, these responses are closely related to pathophysiological processes. The inflammatory process can induce oxidative stress and *vice-versa* through activation of multiple pathways. Therefore, agents with antioxidant and/or anti-inflammatory activities are very useful in the treatment of many pathologies. *Clerodendrum cyrthophyllum* Turcz, a plant belonging to the Verbenaceae family, is used in Vietnamese traditional medicine for treating migraine, hypertension, inflammation of the throat, rheumatic arthritis. Despite its usefulness, studies on its biological properties are still limited. In this study, anti-oxidant and anti-inflammatory properties of an ethanol extract from leaves of *C.cyrtophyllum* (CCEE) were evaluated. In an *in vivo* anti-antioxidant test, 3 day-post fertilization (dpf) zebrafish larvae were treated with CCEE at 5, 20 and 40 µg mL-1 for 1 h and then exposed to 10 µM CuSO4 during 20 min to induce oxidative stress. Fluorescent probes were used to detect and quantify oxidative stress by measuring the fluorescent intensity (FI) in larvae. At 5 and 20 µg mL-1, the CCEE displayed a significant reduction of FI when compared with control group, indicating that it had profound antioxidant effects, reducing or preventing oxidative stress from CuSO4. Moreover, an experiment on 3 dpf zebrafish larvae treated with CCEE at 5, 20 and 40 µg mL-1 for 1 h and then exposed to 10 µM CuSO4 for 4 h showed that CuSO4 elicited a general stress response by the upregulation of *hsp70* and *gadd45bb,* involved in inducible DNA damage repair. But, the co-administration of CCEE protected zebrafish larvae against oxidative damage of CuSO4 through a down-regulation of *hsp70* expression and the upregulation of glutathione S-transferase genes *gstp1* and *gstp2*. To evaluate the anti-inflammatory properties of CCEE, a similar experiment was designed, using 10 µM CuSO4 to stimulate inflammation reaction. After 4 and 24 h of CuSO4 exposure, the expression of genes related to inflammatory process was analyzed in zebrafish larvae. Due to the copper accumulation in zebrafish tissues, the damage and oxidative stress were exacerbated overtime, resulting in the upregulation of genes related to inflammatory process such as *COX-2, PLA2*, *C3a*, pro- and anti- inflammatory cytokines (*IL-1, TNF-α; IL-10* respectively). However, the association of CuSO4 with CCEE reduced significantly *COX-2, PLA2, C3a, IL-1*. Taken together, the results suggested that CCEE has potent anti-oxidant and anti-inflammatory activities and may be useful in the treatment of various inflammatory diseases.

KEYWORDS
Anti-inflammation, oxidative stress, anti-oxidant, CUSO4, zebrafish larvae, *Clerodendrum cyrthophyllum*

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O-018

IDENTIFICATION OF PRIMORDIAL ORGANIZED LYMPHOID STRUCTURE IN THE SPLEEN OF TELEOST FISH.

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ABSTRACT

Induction of immune responses in higher vertebrate species occur within organized lymphoid structures (e.g. lymph nodes, Peyer's patches). These structures probably emerged throughout evolutionary time with the goal to maximize encounters between antigens, antigens-presenting cells and lymphocytes. The current dogma states that Teleost fish lack such structures and thus, it is ill-understood how adaptive immune responses develop in these species. This knowledge in fish has been held back for decades due to the lack of reliable antibody reagents with the capacity to recognize the different subsets of the fish leukocytes involved in the induction of adaptive immune responses.

Over the last few years our laboratory have produced several antibody reagents that recognize different subsets of B and T cells. These antibodies have enabled us to start addressing the mechanism and cells involved in the generation of adaptive immune responses in fish. To understand how immune responses are induced in teleost lymphoid organs, we infected fish with *Ichthyophthirius multifiliis,* a parasite that induces both systemic and mucosal antibody responses in Rainbow Trout. Immune responses were followed with a panel of trout anti-leukocyte antibodies using flow cytometry as well as immunofluorescence, and 3D confocal microscopy, which enabled the analysis of the kinetics and spatial organization of proliferative and resting B and T lymphocytes respectively. Overall, our results identified the spleen as the major site for CD4+ T and IgM+ B cell proliferation in systemic lymphoid organs upon infection. The proliferating splenic IgM+ B cells were frequently observed as clusters in the vicinity of melano-macrophage centers. Moreover, in these areas we observed aggregates of B and T lymphocytes with a loose organized structure reminiscent of the cellular architecture frequently associated with tertiary lymphoid organs. Laser dissection microdissection of these areas has enabled us to start evaluating both the transcriptome and immunoglobulin repertoire that characterize these structures upon infection. In conclusion, these data offer important clues regarding the cellular structures and mechanisms by which adaptive immune responses develop in teleosts, and suggest the existence of primordial semi-organized lymphoid tissue in the spleen in which such responses are induced.

KEYWORDS
IgM+ B cell, CD4+ T cell, adaptive immune response, organized lymphoid structure, semi-organized lymphoid structure

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O-019

IMMUNOMODULATORY ROLE OF SECRETED IGD IN RAINBOW TROUT

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ABSTRACT
In mammals, IgM and IgD are co-expressed on the surface of naive B cells, which, upon antigen binding, down regulate IgD expression, accounting for the gradual disappearance of IgD from the cell surface of activated cells that goes along with somatic hypermutation and class-switch DNA recombination to diversify the Ig gene repertoire. Nevertheless, IgD seems to play a relevant role in the human upper respiratory tract where IgD-secreting cells are found. In humans, this secreted IgD has been shown to play a role in immune surveillance through the activation of basophils and mast cells. In fish, IgD+IgM- B cells have been identified in rainbow trout gills and catfish blood. In both cases, these cells have been shown to have a plasmablast phenotype and are thought to be responsible for the production of secreted IgD found in the serum of these species. However, the immune role of this secreted IgD in teleost remains uncertain. In this study, we demonstrate an immunomodulatory role for secreted IgD. Stimulation of kidney leukocytes with IgD purified from rainbow trout serum provoked a strong immunomodulatory effect, in which the transcription of many immune genes was significantly up-regulated, including pro- and anti-inflammatory cytokines and complement factors. Flow cytometry was used to characterize the cell population which was binding and responding to secreted IgD among head kidney leukocytes. Finally, experiments aimed at characterizing the interplay between secreted IgD and microbiota were also undertaken. Our results demonstrate that as in mammals, secreted IgD has an immunomodulatory role regardless of its antigen specificity.

KEYWORDS
Rainbow trout, secreted IgD, kidney leukocytes, immunomodulation, microbiota.

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O-020

RAINBOW TROUT CD38 DEFINES A SUBSET OF B CELLS IN RAINBOW TROUT

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ABSTRACT

CD38 is a member of the ribosyl cyclase family. It is a multifunctional molecule that functions both as a transmembrane signaling receptor and as an ectoenzyme with important roles in cell adhesion, calcium regulation and signal transduction. In mammals, although expressed in different leukocyte subsets, within the B cell linage, CD38 is usually considered an activation marker. In humans, CD38 is transiently expressed on early lymphocyte precursors, is lost on mature lymphocytes and is consistently expressed on terminally differentiated plasma cells. In the present work, we have identified a homologue of CD38 in rainbow trout (Oncorhynchus mykiss). Using a newly developed monoclonal antibody against this CD38 molecule, the presence of CD38+ populations among IgM+B cells and IgM- leukocytes has been investigated in different rainbow trout lymphoid and mucosal tissues through flow cytometry and immunofluorescence techniques. Moreover, after cell sorting of the different populations identified, the molecular expression profile of each subset has also been determined. Finally, the capacity of different cytokine combinations to regulate the percentage of CD38+ populations has also been established. This study contributes to further understanding B cell differentiation processes in teleost, through the identification of novel cell subsets among B cells.

KEYWORDS

Rainbow trout, CD38, B cells, plasma cells, IgM

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O-021

KINETICS OF RAINBOW TROUT B CELL RESPONSE AGAINST VHSV: NEW INSIGHTS FROM HEAD KIDNEY ANTIBODY REPERTOIRE STUDIES

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ABSTRACT
Upon infection, B-lymphocytes expressing antibodies specific for the intruding pathogen develop clonal responses triggered by pathogen recognition via the B-cell receptor. In teleost fish, in absence of lymph nodes the kinetics and anatomy of B-cell responses remain poorly characterized.
After B cells encounter their specific target, they differentiate into Ab secreting cells probably in spleen or kidney, and it is believed that mature plasma cells migrate and persist in the anterior kidney. Here, we undertook a comparative analysis of the variable heavy chain (VH) domain repertoires of the IgM and IgT in the head kidney of isogenic rainbow trout (*Oncorhynchus mykiss*) lines after primary infection, vaccination and boost with an attenuated strain of the rhabdovirus Viral Hemorrhagic Septicemia Virus (VHSV). We used a barcoded IgH cDNA sequencing approach to characterise the modifications of the antibody repertoire, through the analysis of VH usage in expressed V(D)J rearrangements, clonal diversity, frequency of clonotypes, clonotype distribution and sharing between individual fish and treatments, which is determined by convergent response to Ag and probability of generation by the recombination process. We found extensive modifications implicating most VH families one month after primary infection. In contrast, only
modest changes in terms of repertoire diversity and composition were observed 5 months postvaccination with the attenuated VHSV vaccine. A boost performed 5 months post vaccination induced additional alterations of the kidney IgH repertoire detected after one week, but they faded after one month. The IgM public response implicating VH5 and JH5, that we previously described in spleen, was again observed in all infected fish confirming its presence among B cells from the head kidney and its strong correlation with the response against the virus. Our results provide insights about the amplitude of the primary and secondary B cell response to VHSV infection in the tissue in which plasma cells and memory cells accumulate. Together with a standardisation effort of salmonid Ig genes annotation, these data pave the way for a better monitoring of B cell response kinetics in lymphoid tissues, an essential step to understand B memory mechanisms in fish.

KEYWORDS
Antibody. B cells. Repertoire. Antiviral immunity. Memory.

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O-022

THE DIFFERENTIAL REGULATING ROLES OF MTOR SIGNALING ON IGM+ AND IGT+ B CELLS IN SYSTEMATIC AND MUCOSAL IMMUNE RESPONSES OF RAINBOW TROUT

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ABSTRACT
mTOR is an evolutionarily conserved serine/threonine kinase that plays a key role in cell growth and metabolism by sensing various environmental cues. In mammals, mTOR signaling functions as a metabolic checkpoint to influence the immune responses of T cells, B cells, dendritic cells, and other immune cells. However, the regulating role of mTOR signaling on fish cellular and humoral immunity is much less known. Our previous studies indicate that mTOR signaling also functions as the sensor and regulator of fish immunometabolism during pathogenic infection. To address the regulating roles of mTOR signaling on the immune responses of B cells in fish, we used rapamycin to inhibit the mTOR signaling of rainbow trout (*Oncorhynchus mykiss*) both *in vitro* and *in vivo*. After 3 days incubation *in vitro*, the relative percentages of IgM+ and IgT+ B cells within the whole isolated leukocytes were significantly decreased after rapamycin treatment. mTOR signaling of rainbow trout was significantly inhibited by rapamycin treatment, indicated by the inactivation of phospho-TOR and phospho-S6. mTOR signaling inhibition in rainbow trout *in vivo* resulted in poor growth performance and decreased feed efficiency, accompanied with which was the altered metabolism of glucose, glutamine and fatty acid. Compared to their counterparts in the control group, IgM concentration in the serum was significantly decreased, while IgT concentration in the gill mucus was significantly decreased after rapamycin treatment. Crucially, the relative percentages of IgM+ and IgT+ B cells in the head kidney fell by ~80% and ~20%, respectively, while the relative percentages of both IgM+ and IgT+ B cells in the spleen fell by ~50 % when mTOR signaling was inhibited. However, the relative percentages of IgM+ and IgT+ B cells in the gill fell by ~25% and ~75% after rapamycin treatment, respectively. Importantly, further studies indicated that, accompanied with the inhibition of mTOR signaling, both the proliferation of IgM+ B cells in the head kidney and the proliferation of IgT+ B cells in the gill were significantly inhibited. These data revealed the differential regulating roles of mTOR signaling on IgM+ and IgT+ B cells in fish systematic and mucosal immune responses for the first time, and further studies should be conducted to reveal the regulating mechanism of mTOR signaling on fish humoral immunity during pathogenic infection.

KEYWORDS
mTOR signaling, B cells, rainbow trout, gill, head kidney.

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O-023

EVIDENCES FOR THE IMMUNOMODULATION BY THE MELATONIN HORMONE IN PIKEPERCH *Sander lucioperca*

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ABSTRACT

The melatonin hormone is produced and secreted by the pineal gland during the dark phase of the photoperiod. It hence provides information such as time of the day and season for cells and organs. As in mammals, melatonin in fish is known to act on important physiological functions, including thermoregulation, reproduction and development. However, while well described in mammals, few studies have investigated its potential role on immune functions in teleost. In pikeperch, we defined in previous experiments a potential dual action of cortisol and melatonin hormones on immune defenses. In addition, we characterized daily cyclic activities of humoral innate immune markers that are correlated with the cyclic release of melatonin by the pineal gland.

Nocturnal peak of melatonin production and release is directly proportional to the length of the night and hence provides a direct transduction of night length. In order to deepen our knowledge on the immune modulation by the melatonin hormone, we hypothesized that changing photoperiod influences the fish immune functions through the modulation of melatonin synthesis. The study thus investigated the effects of two natural photoperiod regimes simulating the fall and the spring in western Europe on melatonin secretion, stress and immune markers.

Daily cyclic activities were observed for plasma melatonin and cortisol, but also for several innate immune markers, including lysozyme, peroxidase and complement activities in plasma and phagocytic activity in spleen. Nocturnal plasma melatonin values were influenced by the seasonal simulated photoperiods with progressive increase or decrease for the photoperiods simulating the fall and the spring respectively. No photoperiod effect was detected on cortisol release. Moreover, the exposure to the fall-simulated photoperiod induced several effects on immune markers, including increases in lysozyme, peroxidase and complement activities. Analyses of immune-relevant gene expression are ongoing. Our results bring an additional evidence supporting the potential immunomodulatory action of the melatonin hormone in teleosts with a stimulation of the innate immunity following the increase in melatonin production in response to the fall-simulated photoperiod.

KEYWORDS

Melatonin; photoperiod; immune system; circadian axis; pikeperch

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O-024

MODIFICATIONS OF MUCOSAL AND SYSTEMIC ANTIBODY REPERTOIRE AFTER ERM NASAL VACCINATION IN RAINBOW TROUT.

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ABSTRACT
Bony fish have a dedicated mucosal immune system which comprises immunologically heterogeneous microenvironments armed with innate and adaptive immune components. In rainbow trout (*Oncorhynchus mykiss*), a nasopharynx-associated lymphoid tissue (NALT) was recently described as a diffuse network of myeloid and lymphoid cells located in the olfactory organ of fish. Teleost NALT presents IgM and IgT B cells in equal proportions and nasal mucus contains secreted IgM and IgT. Several studies have demonstrated that nasal vaccination is a very effective mucosal route to stimulate adaptive immune responses and high levels of protection against viral and bacterial pathogens in fish. However, the mechanisms underlying the observed protection are not well understood. We applied a barcoded 5´RACE IgH cDNA sequencing approach to investigate the structure of the systemic and mucosal rainbow trout immunoglobulin repertoire. Its analysis in control trout suggests different structures of IgM and IgT spleen and NALT repertoire, with restricted repertoire diversity in NALT. Nasal and intraperitoneal vaccination with enteric red mouth (ERM) vaccine also revealed unique dynamics of IgM and IgT repertoires at systemic and mucosal sites and the remarkable ability of nasal vaccines to induce spleen Ig responses. Our findings provide an important immunological basis for the effectiveness of nasal vaccination in fish and other vertebrate animals and will help the design of future nasal vaccination strategies.

KEYWORDS
NALT, B cells, Immunoglobulin, Repertoire, Vaccine.

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O-025

UNDER CONTROL: 20 IRAK3 VARIANTS REGULATE TOLL-LIKE- AND INTERLEUKIN-1-RECEPTOR SIGNALLING IN RAINBOW TROUT

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ABSTRACT
The immune system of vertebrates enables the rapid and very efficient defence against microorganisms and viruses. Shortly after the detection of pathogens, amplifier mechanisms multiply various destructive activities, which may, however, also be directed against the host itself. An arsenal of inhibitory factors controls therefore the duration and extent of the immune response, restricts pathological events and restores homeostasis. For these reasons, it is essential that endogenous immune regulators are integrated in efficient health concepts in aquaculture.
However, immune inhibitors in teleost fish are still poorly explored, also due to the fact that the teleostean repertoire of immune inhibitors is more complex than the mammalian one. We found that the inhibitory kinase interleukin-1 receptor-associated kinase 3 (*irak3*) is present in more than 20 isoforms of varying length and nucleotide composition in rainbow trout. We elucidated the underlying genetic causes for this striking Irak3 diversity and profiled the expression of all *irak3* variants in healthy and infected rainbow trout. The obtained data revealed that the truncated *irak3* variants are expressed to a much greater extent than the full-length variants. The overexpression of selected full-length and truncated Irak3 variants in different cell models showed that the individual isoforms modulate the basal as well as the pathogen-induced activity of NF-kappaB with different efficiency. Confocal microscopy showed that the overexpression of the truncated Irak3 variant was associated with massive cell death, in contrast to the full-length variant. Based on these different observations, we assume that the multiple Irak3 variants do not represent sheer abundance. Rather, we hypothesize that different Irak3 variants could integrate specificly into the different cascades mediated by IL1R1 and more than a dozen TLRs. Certain Irak3 isoforms might suppress the inhibitory functions of their paralogs to steer the immune response from a suppressed to a reinducible state. Further analyses are on the way to test the hypotheses using suitable cell models and appropriate knock-out or knock-in techniques.

KEYWORDS
Inhibitory factors; Innate Immunity; IRAK-3; Salmonids; Toll-like receptor signalling

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O-026

EVIDENCE OF IgD-SECRETING PLASMABLASTS AND MUCOSA SPECIFIC IgD MOLECULAR SIGNATURES IN TELEOST GILLS AND GUT

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ABSTRACT

IgD is an ancient immunoglobulin for which many aspects of its regulation and function remain unclear. Although usually expressed on the surface of mature B cells along with IgM, a small subset of B cells undergo an unconventional IgM-to-IgD class switch and differentiate to IgD+IgMplasmablasts/plasma cells. In mammals, these cells are mostly found in nasopharyngeal lymphoid tissues and rarely in the intestine, although recent evidence suggests a role for IgD in gut immunity.

In teleost fish, a subset of IgD+IgM- B cells was previously reported in rainbow trout gills and in catfish blood. Here, we report that IgD+IgM- B plasmablasts also constitute a major B cell subset in the intestinal mucosa of rainbow trout. Thus, although IgD+IgM+ B cells represent almost 87% of the IgT- B cell subsets in the spleen, IgD+IgM- B cells accounted for 83% of IgT− B cells in the gut. A complete repertoire analysis of IgD in comparison to IgM and IgT performed in gills and gut as well as in spleen, confirmed the clonal expansion of IgD in these two mucosal sites but not in spleen. Remarkably IgD sequences in gills and gut share a common VDJ segment usage and had quite similar mutation profiles, different from those found in the spleen. Our data demonstrates that IgD-secreting plasmablasts represent a major B cell subset in rainbow trout gills and gut and that the IgD sequences derived from the clonal expansion of specific B cells found in these two tissues differ greatly from the IgD found in spleen that is mostly found on the cell surface in association with IgM.

KEYWORDS

Rainbow trout, IgD, gills, gut, repertoire

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O-027

DEVELOPMENTAL IMMUNOTOXICOLOGY: WHAT UNDERLIES THE CRITICAL WINDOWS OF EXPOSURE?

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ABSTRACT

Endocrine disruptors in general and oestrogenic compounds in particular have been widely investigated in view of their effects on several physiological processes and, therefore, their ecotoxicologic relevance. The modulation of the immune system by oestrogens has increasingly sparked interest in the research community, that previously had been mainly centered on the reproductive effects of these hormones. In fact, since the industrialization an increasing variety of endocrine disruptors, such as oestrogenic endocrine disruptors, are retrieved in the environment. These oestrogenic endocrine disrupting chemicals (EEDCs) have also been suggested to increase the prevalence of autoimmune diseases and cancer. With regard to the high degree of similarities in the immune system of jawed vertebrates as well as the conserved immunomodulatory roles of oestrogen, environmental EEDCs possibly have the capacity to alter immune system functions of teleost fish, which may impair their capacity to fight infectious diseases and eventually may contribute, together with overfishing, to wild stock losses. Importantly, the most deleterious effects of EEDCs, both in mammals and teleosts, appear to arise when the exposure occurs during specific periods of the immune system ontogenesis, commonly referred as critical windows of exposure. However, in mammals and especially in teleost fish, these stages of the immune system development as well as the EEDC-action remain to be fully identified and characterised. The concept and the importance of developmental immunotoxicity is presented by addressing the mechanisms of oestrogenic regulation and the mode of action of EEDCs from an immunological perspective. Emphasis is given to the critical windows of development of the immune system during which EEDCs may alter the immune system development and function with long-term consequences on immunocompetence. Results from different classes of vertebrates are compiled, highlighting studies on teleost fish and their relevance for the human immune system. Additionally, new results on the effects of environmentally relevant concentration of exogenous estradiol exposure during European sea bass (*Dicentrarchus labrax*) development will be presented with regard to oestrogen's ability to trigger effects on immunocompetence, contributing to fill the gaps on vertebrate immunotoxicology.

KEYWORDS
Developmental immunotoxicity; Immune system; Critical windows; Endocrine disruptors; Oestrogens

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O-028

CONTRASTING REACTIVITY OF LYMPHOCYTE SUBSETS TO IL-15, IL-15L AND IL-2 IN RAINBOW TROUT

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ABSTRACT

Interleukin (IL)-2 and IL-15 are used as immunostimulants in human medicine since they are known to induce activation, proliferation and maintenance of several lymphocyte subpopulations. In addition to IL-2 and IL-15, rainbow trout possess a third closely related functional cytokine IL-15like (IL-15L). Our previous studies have shown that the soluble form of the corresponding receptor (sIL-15Ra) enhances the secretion and/or stabilization of IL-15 and IL-15L *in vitro*. Therefore in this study, chimeric recombinant proteins of IL-15 and IL-15L with sIL-15Ra (IL-15-RLI and IL-15L-RLI, respectively) as well as IL-2 (as a free cytokine) were produced by using a baculovirus/insect cell system. These cytokines were then subjected to *in vitro* functional studies. For this, purified recombinant proteins were added to four different flow-sorted thymocyte sub-populations: CD4-Single Positive (SP), CD8-SP, CD4/CD8-Double Positive (DP) and Double Negative (DN) and to three lymphocyte sub-populations (CD4-SP, CD8-SP and DN) from intestine and spleen applying anti-CD8 and anti-CD4 monoclonal antibodies. Stimulated lymphocytes were then subjected to Western blot analysis to detect phosphorylation of STAT5, a molecule that can be found downstream the IL-2/15Rbg chain signal transduction cascade, as a measure of lymphocyte activation. In addition, whole splenocytes were individually incubated with the recombinant cytokines and subjected to RT-qPCR analysis. A contrasting reactivity of lymphocytes to the three recombinant cytokines was observed in lymphocyte subsets from different lymphoid organs. While all of the tested lymphocyte subsets except DP thymocytes were activated by IL-15-RLI, DP thymocytes were only activated by IL-2 which stimulated all thymocyte subsets as well as the CD4-SP and CD8-SP splenocytes. A clear stimulatory effect of IL-15L-RLI was observed in DN thymocytes while DN and CD8-SP splenocytes were only activated at very high concentrations. RT-qPCR analysis confirmed the contrasting stimulatory effect of IL-15 and IL-15L chimeric proteins with IL-15Ra. In trout splenocytes, IL-15-RLI induced type 1 immune gene expression such as *IFNg* and *Perforin*, but did not induce type 2 immune gene expression such as *IL-4/13*. In contrast, IL-15L-RLI induced type 2 immune gene expression but did not enhance type 1 immune gene expression. These results reveal the distinctive powers of the IL-2/15/15L family cytokines, and hold great promise for their practical use as well as for their capacity to help unravel the different arms of the fish immune system.

KEYWORDS
Rainbow trout, IL-2 cytokine family, adaptive immunity, lymphocytes, recombinant proteins

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O-029

MACROPHAGE POLARIZATION IN FISH TRANSCRIPTIONAL PROFILES AND METABOLIC CHANGES

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ABSTRACT
Macrophages of higher vertebrates can display a range of functional phenotypes, while the chief M1 and M2 activation states appear to operate under the guidance of primordially-conserved principles. We have been studying the evolutionary conservation of these M1 and M2 macrophage activation states in teleost carp, mainly by measuring functional responses such as nitric oxide production (M1) and arginase activity (M2). However, the picture of M1 and M2 activation states in teleosts is still far from complete. To complement our understanding of teleost macrophage polarization we first studied activation-state specific gene expression profiles through an unbiased whole transcriptome approach in addition to functional assays. Secondly, we studied the conservation of bioenergetic and metabolic pathways paramount to activation-state specific functions.
Here we report differential transcriptional profiles for M1 (LPS stimulated) and M2 (exogenous cAMP stimulated) carp macrophages and discuss the conservation of these profiles, which include multiple conserved markers. In addition, we show an enhanced M1 profile when IFN-γ is combined with LPS.
Although essential to direct and support macrophage activation-state specific functions, conservation of bioenergetic and metabolic pathways have not been studied in detail in polarized carp macrophages. Generally, mammalian M1 macrophages show relatively high glycolysis rates while M2 macrophages are geared towards oxidative phosphorylation to generate energy. We studied whether the enhancement of these specific energy metabolism pathways is conserved. We optimized for carp macrophages the determination of cellular oxygen consumption rate (OCR) as a measure for oxidative phosphorylation, and the determination of extracellular acidification rate (ECAR) as a measure for glycolysis, using the Seahorse real-time Mito Stress Test. We have gained insight in the energy metabolism pathways utilized by carp macrophages driven to M1 or M2 activation states by using specific parameters measured with this test. Both our whole transcriptome approach and assays to measure bioenergetic and metabolic pathways provide valuable additions to studies addressing the evolutionary conservation of M1 and M2 macrophage activation states.
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KEYWORDS
Macrophage, polarization, transcriptome, metabolism, immunometabolism

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O-030

ADVANTAGES AND DISADVANTAGES OF ZEBRAFISH AS A MODEL OF INFLAMMATION

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ABSTRACT
Zebrafish (*Danio rerio*), largely used as a model for studying developmental processes, has also emerged as a valuable system for modelling human disease. Zebrafish possesses a complex immune system comparable to those of mammalian models. Nevertheless, 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 inflammatory response. This is the case of the of the complement component *c3* with 8 different genes in the zebrafish genome and different functions. This aspect, that could be considered an inconvenient, contrasts with the clear advantages that this model offers. 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. Also, we demonstrate how the genomic responses of adult zebrafish tissues can effectively reproduce the mammalian inflammatory process induced by acute endotoxin stress. Immune signalling has been well conserved throughout evolution and zebrafish and mammal genomic responses after lipopolysaccharide stimulation are highly correlated. Therefore, we confirm that zebrafish is an ideal model to study the basic mechanisms of inflammation and to model human inflammatory diseases.

KEYWORDS
Zebrafish, inflammation, imaging, complement, transcriptome

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O-031

VIRAL RESISTANCE AND INTERFERON SIGNALLING IN SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION (STAT)-1 AND STAT2 KNOCKOUT SALMONIDS CELLS

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ABSTRACT

Interferons (IFNs) belong to a group of cytokines specialised in the immunity to viruses. Upon viral infection, type I IFN is produced and alters the transcriptome of responding cells through induction of a set of Interferon Stimulated Genes (ISGs) with regulatory or antiviral function, resulting in a cellular antiviral state. Fish genomes have both type I IFNs and type II IFN (IFNγ), but type III (λ) IFN has not been identified in fish. The mechanisms of the downstream signalling remain partly undefined. In mammals, members of the Signal Transducer and Activator of family of transcription (STAT) factors are responsible for the transmission of the signal from cytokine receptors, and STAT2 is required for type I but not type II IFN signalling. In fish, the role of STAT2 in IFN signalling remains unclear. Using CRISPR/Cas9 genome editing, we generated two Chinook salmon (*Oncorhynchus tshawytscha*) cell lines with transcription factors STAT1 and 2 knocked out. GS2 and GS1A are stat2-/- and stat1a1-/- stat1a2-/- stat1b1+/+ stat1b2+/+, respectively. In these cell lines, the induction of ISGs by stimulation with a recombinant type I IFN is completely obliterated as evidenced by RNA-seq analysis of the transcriptome and/or qPCR gene expression profiling in comparison with the wild type parental cell line. In contrast, the type II IFN signalling pathway is obliterated only in GS1A but not in GS2. Despite a complete absence of ISGs induction, the GS2 and GS1A cell lines still have a remarkable ability to inhibit viral replication. Therefore, other STAT1/2-independent pathways may be induced by the viral infection, potentially illustrating the robustness and redundancy of the innate antiviral defences in fish.

KEYWORDS

Chinook salmon, CRISPR/Cas9, STAT2, STAT1, interferon signalling

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O-032

PHYLOGENY AND EXPRESSION OF THE TETRASPANIN CD9 IN SALMONID CELL LINES IN RESPONSE TO INTERFERON STIMULATION

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ABSTRACT
CD9 is a member of the cell membrane associated tetraspanin family and has been shown to have a wide array of functions, including promotion of MHC clustering, antigen presentation, T cell activation, cell adhesion, motility, growth and differentiation, signal transduction, tumor formation and egg/sperm fusion. CD9 is ubiquitously expressed in mammalian tissues and its roles are cell type dependent. CD9 is a typical interferon stimulated gene and further associated with MHC II and the immune system and inflammation in general, as has been shown in mammals and to a lesser extent in fish. In mammals, some viruses, such as influenza, coronavirus and hepatitis C, exploit CD9 for exit of new virus particles from host cells. In contrast, increased expression of CD9 can limit HIV-I virus budding.
Due the limited knowledge of the involvement of CD9 in immune system responses in fish, we explored the phylogeny and expression of this gene in salmonids. We found 6 paralogues, which can be further organized into three distinct clades. We termed these clades CD9a, CD9b and CD9c, each of which include two paralogues reflecting the salmonid specific whole genome duplication. CD9a and CD9b are closely related and have the greatest sequence homology with the mammalian single copy gene of CD9, indicative of the teleost specific whole genome duplication. The CD9c clade is very distinct to CD9a and CD9b in sequence identity and further shows little sequence homology with the mammalian CD9, therefore could be an ancestral form of CD9 that was subsequently lost in all other vertebrate classes.
We investigated the expression of the different paralogues in embryonic chinook salmon cells (CHSE) stimulated with interferon type I, an inducer of the antiviral pathways in fish.
The paralogues of clade CD9c were highly inducible by interferon stimulation, whilst CD9a and CD9b appeared to be non-responsive. The specific inducibility of the ancestral CD9c clade to interferon type I highlights the unique immune responses in teleost. The presence of 6 paralogues organized in three clades may also reflect the diversity of roles this gene has been implicated in. In future, we aim to explore the expression of CD9, especially the putatively immune system relevant clade CD9c, in different cell types at baseline and in response to virus stimulations.
This study contributes to a better understanding of CD9 involvement in immune system responses and how the gene is related to the antiviral interferon type I response. As CD9 has been shown to be important for the replication of certain viruses in mammals, this could be explored for fish viruses and potentially used as an anti-viral target.

KEYWORDS
Tetraspanins, Salmonid, Interferon signaling, Antiviral immune response, Phylogeny

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O-033

GENOMICS FOR THE UNDERSTANDING OF THE HOST-PATHOGEN INTERACTION: THE CASE OF THE ATLANTIC SALMON AND *Piscirickettsia salmonis*

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ABSTRACT
During an infection both host and pathogen undergo a deep transcriptomic remodeling that will orchestrate either the pathogen clearance or host infection. These changes involve both the regulation of protein coding genes (mRNA) and non-coding RNAs (ncRNAs) elements, such as lncRNAs and miRNAs. Thus, knowing how these elements are modulated can reveal key aspects about hostpathogen interaction. Through RNA-seq, miRNA-seq and dual RNA-seq, we explored the coding and non-coding transcriptional response in Atlantic salmon infected with the intracellular bacterium Piscirickettsia salmonis. Differential expression analysis revealed that fish respond to P. *Salmonis* infection through modulation of different coding genes associated with immunity, clathrin mediated endocytosis and iron metabolism responses. In addition, a strong response associated with ncRNAs was also evidenced. Our results suggested that these ncRNAs might fulfilling key regulatory roles in the response of the Atlantic salmon to *P. salmonis* infection. On the other hand, bacteria transcriptomic response was associated with a large number of genes involved in amino acid metabolism. Genome wide comparison and in vitro studies evidenced a metabolic dependency of *P. salmonis* on salmon amino acids. Based in our results, we propose that amino acids might be an important component of the nutritional immunity triggered by the Atlantic salmon to cope with *P. salmonis* infection. Overall, our results evidence how genomics can lead us to the understanding of novel means of interaction between host and pathogens in marine models.

KEYWORDS
Dual RNA-Seq, Piscirickettsia salmonis, Atlantic salmon, Nutritional immunity, metabolic dependency, amino acids.

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O-034

IN VITRO RAINBOW TROUT TRANSCRIPTOME REVEALS IMMUNE EVASION ASSOCIATED WITH HIGHER VIRULENCE OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

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ABSTRACT
Rainbow trout pathogenic freshwater viral haemorrhagic septicaemia virus (VHSV) emerged from an ancestral marine virus, however the pathogenic mechanism of the virulent freshwater VHSV remains unknown. In the present work, the transcriptome of RTG-2 cells inoculated with two pathogenic (J167 and DK-5131) and two non-pathogenic (96-43/8 and 1p49) isolates were analyzed at 3, 6, and 12 hours and compared to control samples using RNA-seq. Although VSHV isolates showed the same pattern of viral replication, the transcriptomic profiles in RTG-2 cells were dramatically different between pathogenic and non-pathogenic isolates, revealing a lack of sensing of the viral replication in cells inoculated with both pathogenic VHSVs at early stages of infection. Functional annotation analysis of differentially-expressed genes between non-pathogenic VHSV and controls revealed an enrichment of pathways involved in the defense to biotic stimulus and metabolic processes (strong up-regulation of genes), and lipid metabolism and cell cycle (down-regulation of genes) In contrast, cholesterol and cytoskeleton mobility pathways were enriched (up-regulation of genes) by both pathogenic VHSV. Furthermore, an increasingly higher number of GRP78/BiP transcripts in cells inoculated with the pathogenic VHSVs suggests a role of the unfolded protein response in the VHSV immune evasion.

KEYWORDS
Rainbow trout, VHSV, transcriptome, RNA-Seq, immune evasion, host-pathogen interaction

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O-035

STUDIES INTO B-GLUCAN RECOGNITION IN FISH SUGGESTS A KEY ROLE FOR THE C-TYPE LECTIN PATHWAY

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ABSTRACT

Immune-modulatory effects of β-glucans are generally considered beneficial to fish health. Despite the frequent application of β-glucans in aquaculture practice, the exact receptors and downstream signalling remains to be described for fish. In mammals, Dectin-1 is a member of the C-type lectin receptor (CLR) family and the best-described receptor for β-glucans. In fish genomes, no clear homologue of Dectin-1 could be identified so far. Yet, in previous studies we could activate carp macrophages with curdlan, considered a Dectin-1-specific β-(1,3)-glucan ligand in mammals. It was therefore proposed that immune-modulatory effects of β-glucan in carp macrophages could be triggered by a member of the CLR family activating the classical CLR signalling pathway, different from Dectin-1. In the current study, we used primary macrophages of common carp to examine immune modulation by β-glucans using transcriptome analysis of RNA isolated 6 h after stimulation with two different β-glucan preparations. Pathway analysis of differentially expressed genes (DEGs) showed that both β-glucans regulate a comparable signalling pathway typical of CLR activation. Carp genome analysis identified 239 genes encoding for proteins with at least one C-type Lectin Domains (CTLD). Narrowing the search for candidate β-glucan receptors, based on the presence of a conserved glucan-binding motif, identified 13 genes encoding a WxH sugar-binding motif in their CTLD. These genes, however, were not expressed in macrophages. Instead, among the β-glucan-stimulated DEGs, a total of six CTLD-encoding genes were significantly regulated, all of which were down-regulated in carp macrophages. Several candidates had a protein architecture similar to Dectin-1, therefore potential conservation of synteny of the mammalian Dectin-1 region was investigated by mining the zebrafish genome. Partial conservation of synteny with a region on the zebrafish chromosome 16 highlighted two genes as candidate β-glucan receptor. Altogether, the regulation of a gene expression profile typical of a signalling pathway associated with CLR activation and, the identification of several candidate β-glucan receptors, suggest that immune-modulatory effects of β-glucan in carp macrophages.

KEYWORDS
β-glucan, primary macrophage, transcriptome analysis, C-type lectin-like domain, cyprinidae

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O-036

THE IMMUNE PROTEOME OF THE ZEBRA MUSSEL DECIPHERED BY DEEP PROTEOGENOMICS.

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ABSTRACT

Bivalve immune system modulation appears to be a relevant strategy in environmental risk assessment. Indeed, immune system is known to be sensitive to different environmental and anthropogenic stresses. To date, the immune system of marine bivalves is well documented in comparison to continental bivalves. Among them, the freshwater mussel *Dreissena polymorpha*, a non-model organism, represents the counterpart of the marine mussel in ecotoxicological studies. While cellular responses of hemocytes are well characterized for *D. polymorpha*, the molecular immune mechanisms remain relatively scared. In order to get insights into the immune proteome of the zebra mussel, proteogenomics was conducted on both hemocytes and plasma compartment of this non-model species. This strategy, combining transcriptomic sequences with mass spectrometry data acquired on proteins was relevant since 3,227 proteins were identified, which represent the largest protein inventory for this sentinel organism. Functional annotation and gene ontology (GO) analysis performed on the identified proteins described the main molecular players of hemocytes and plasma in the immune response of *D. polymorpha*. The GO analysis carried out on immune proteins showed that these two hemolymphatic compartments perform closely related and complementary immune functions: in signal transduction, adhesion and cellular mobility but also related to the recognition and elimination of microorganisms. Functional annotation revealed new mechanisms into the immune defence of the zebra mussel. Proteins rarely observed in the hemolymph of bivalves were pinpointed such as natterin-like proteins and thaumatin-like proteins. Furthermore, the high abundance of complement-related proteins observed in plasma suggested a strong implication of the complement system in the immune defence of *D. polymorpha*. This study contributes to a better understanding of the molecular mechanisms involved in immunity of bivalves and paves the way for their use as biomarkers in aquatic ecotoxicology.

KEYWORDS

Hemolymph, bivalve, immunity, non-model organism, proteogenomics

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O-037

SALMONID IGH GENES: FROM GENOMICS TO REPERTOIRE SEQUENCING

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ABSTRACT
Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) represent key species in aquaculture and are important models for the development of fish immunology. As in mammals, the basis of teleost humoral adaptive immune response is the clonal expression by B cells of somatically diversified immunoglobulins (IG), either as membrane bound or secreted in response to infections or immunizations. The IG repertoire sequencing has started to develop both in rainbow trout and in Atlantic salmon, reflecting a growing interest for an accurate and comprehensive description of the response against common pathogens and vaccines. In this context, a unified and standardized nomenclature and classification of IG genes is needed. In addition, these species are of particular interest because their IG loci are complex due to two additional whole genome duplication (WGD), compared to tetrapods: a WGD event that occurred during early teleost evolution, and a recent WGD that is specific to salmonids. This is reflected in the identification of IGH isoloci on two chromosomes. A good quality genome assembly is now available for rainbow trout and Atlantic salmon allowing a fully annotation that provide novel information. Here, we present how an IMGT-based nomenclature, numbering and structural description can be established in the frame of the Inferred Allele Review Committee (AIRC) working group, and how it helps comparing the diversity, the structure and the dynamics of antibody repertoires between fish and mammals.

KEYWORDS
Immunoglobulin, locus, repertoire, nomenclature, Salmonids.

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O-038

METAGENOME ANALYSIS OF INTESTINAL FLORA IN THE IL-17A/F1-KNOCKOUT MEDAKA

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ABSTRACT

In mammals, interleukin (IL)-17A and IL-17F are hallmark inflammatory cytokines, which are expressed by Th17 cells and play key roles in protection against infection and intestinal mucosal immunity. However, although fish IL-17A and IL-17F homologs named as IL-17A/F have been identified, their functional aspects, especially in intestinal mucosal immunity are still poorly understood. In this study, IL17A/F1-knock-out (IL17AF1-KO-) medaka (Oryzias latipes) was established using the genome-editing technique, CRISPR/Cas9 system, and a 7-bp deletion (-7bp) and a 11-bp addition (+11bp) were confirmed in the IL-17A/F1-KO-medaka. After establishing F3 homo KO-medaka (+11bp), we conducted bacterial infection test with Edwardsiella tarda (E381 strain) to compare the defense capability in intestine of IL-17A/F1-KO-medaka to those of wild type (WT) medaka. After 24 hours immersion in freshwater containing 2.1Å~108 CFU/ml E. tarda, the number of bacteria was higher in posterior intestine than in anterior intestine in both WT and IL-17A/F1-KO-medaka. However, after 48 hours, bacterial number in posterior intestine decreased to the same extent as in anterior intestine at the same time. Furthermore, in comparison between WT and IL-17A/F1-KO-medaka, bacterial number of E. tarda in posterior intestine of IL-17A/F1-KO-medaka increased in 24 hours compared to those of WT. In addition, the results of gene
expression in intestine by real-time PCR (qPCR) showed that antimicrobial peptide genes such as G-type lysozyme and transferrin a after infection were significantly down-regulated in IL-17A/F1-KO-medaka compared to those of WT. Furthermore, we performed 16S rRNA-based metagenome analysis to compare changes in composition of intestinal bacterial flora during naïve and infection between IL-17A/F1-KO and WT medaka. As a result of α diversity analysis, under naïve condition, the diversity of bacterial flora was less in the WT medaka than in the KO medaka. After infection, although, the diversity of bacterial flora increased in both KO and WT medaka, bacterial species of WT medaka increased over twice in 24 and 48 hours after infection in comparison to those of naïve group, while there was a 1.5 times increase of bacterial species in IL-17A/F1-KO groups in 24 and 48 hours after infection. Furthermore, in weight-UniFrac analysis, it was revealed that WT and IL-17A/F1-KO group under naïve condition form different clusters. These results suggested that IL-17A/F1 induces a change in the composition of the intestinal bacterial flora in medaka.

KEYWORDS
Interleukin 17, Japanese medaka, Antimicrobial peptide, 16S rRNA-based metagenome, Genome editing

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O-039

DIFFERENTIAL MICROBIOTA AND IMMUNE MODIFICATION IN RAINBOW TROUT WHEN FACING BACTERIAL INFECTION

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ABSTRACT
Abstract must have a maximum of 2700 characters including spaces. In metazoans, the epidermal surface is important to maintain homeostasis of individuals. These epidermis are colonized by bacteria that have co-evolved with the host and that form communities with a complex network of interactions, called the microbiota. Communication between microbiota and the host was made possible by developing a suitable immune system. The microbiota is involved in many crucial functions for the host such as the maturation and stimulation of innate and adaptive immunity and the defense against pathogens by avoiding their colonization. Therefore, it is essential for the host to maintain homeostasis within the microbiota and between its mucosal immune system and the microbiota to keep functionality. However, this communication between those two compounds can be disrupted by various kinds of stressors present in the organism's environment. Such disturbance of this homeostasis is called a dysbiosis and can lead to detrimental, even mortal consequences for the host. Among these stressors, we can find some diseases caused by bacterial infection such as *Aeromonas salmonicida*. This pathogen is the causative agent of furunculosis and lead to important mortality in aquaculture. In this study, we have described the microbiota from different epithelial locations (skin, gills, caudal fin) exposed to a bacterial stressor (*Aeromonas salmonicida achromogenes*) using Next Generation Sequencing (Illumina HiSeq 2500). The hypervariable region V1-V3 16S rRNA gene was sequenced to assess taxonomical composition and structure of different epithelia 6 h and 72 h after a short bacterial bath infection (1 h). We also assessed the immune status of fish after bath infection through qPCRassays. Alpha diversity measurements (inverse Simpson and Shannon index) suggest that the different microbiotas are highly diverse but predominated by few taxa and that the bacterial infection does not affect these indices. On the other hand, beta diversity measurements showed a potential early infection through gills after 6 h,then affecting skin and caudal fin microbiotas. Negative binomial generalized linear model (nb-GLM) highlighted the increase of different opportunistic pathogens such as Aeromonas, Pseudomonas, etc 6 h after bath infection. These observations are consistent with immune assays (cytokines, humoral response, gene expressions) showing a response of the immune system after 6 h followed by a disorder in its functioning. This study suggests that furunculosis not only impair immune system in rainbow trout but also induce dysbiosis leading to the increase of opportunistic pathogens in the bacterial community.

KEYWORDS
Aquaculture, Microbiota, pathogen infection, immune system, *Oncorhynchus mykiss*

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O-040

TLR5M AND TLR5S PLAY OPPOSITE ROLES IN NF-KB PATHWAY IN *Vibrio parahaemolyticus* FLAGELLIN STIMULATION IN ORANGE-SPOTTED GROUPER, *Epinephelus coioides*

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ABSTRACT

A family of Toll-like receptor (TLRs) acts as primary sensors, which detect various microbial components, activate the host innate immune response to eliminate invading pathogens. In mammals, nuclear factor (NF)-κB signaling pathway is critical to the inflammatory cytokines and effectors expression. However, in fish, the bidirectional regulation of TLRs on NF-κB pathway is poorly understood. Here, we investigated the potential molecular mechanism of orange-spotted grouper (*Epinephelus coioides*) TLR5M (*Ec*TLR5M) and TLR5S (*Ec*TLR5S) regulating the NF-κB pathway after *Vibrio parahaemolyticus* flagellin stimulation.

*Ec*TLR5M, a member of the conserved TLR protein family, is involved in specifically recognizing flagellin and activating the NF-κB pathway. After knockdown of *Ec*TLR5M in grouper spleen (GS) cell line, the phosphorylation of IκBα and the expression of downstream cytokines, such as interferon (IFN) -γ2, interleukin (IL) -6 and tumor necrosis factor (TNF) -α, were all significantly suppressed. The overexpression of *Ec*TLR5M induced not only the activation of NF-κB pathway, but also mRNA expression of *Ec*TLR5S.

*Ec*TLR5S, consisting of 17 extracellular leucine-rich repeat domains, is located in the cytoplasm and involved in flagellin recognition. Knockdown of *Ec*TLR5S enhanced the phosphorylation of IKKα/β and IκBα, promoted NF-κB p65 nuclear transport, and augmented the cytokines IFN-γ2, IL-6 and TNF-α mRNA expressions after flagellin stimulation. Consistently with these observations, over-expression of *Ec*TLR5S negatively regulated the NF-κB pathway activation. We verified that the N-terminal (aa 1-254) and C-terminal (aa 514-643) of *Ec*TLR5S are the major functional domains of negative regulation by deletion mutation.

Taken together, *Ec*TLR5M is identified as a positive regulator, activating the NF-κB signaling after flagellin recognition. And for the first time, *Ec*TLR5S is demonstrated as a negative regulator that suppresses flagellin-induced activation of NF-κB, suggesting an important role for *Ec*TLR5S in control of innate immunity homeostasis.

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KEYWORDS
NF-κB; TLR5M; TLR5S; flagellin; cytokine

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O-041

BACTERIAL OUTER MEMBRANE VESICLES OF *Aeromonas salmonicida* INDUCE A PROINFLAMMATORY IMMUNE RESPONSE IN VITRO AND IN VIVO

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ABSTRACT
High mortality rates after bacterial infections cause huge annual losses for the aquaculture industry. As treatment with antibiotics is not an alternative, bacterial vaccines for intramuscular or intraperitoneal injection were developed resulting in protection but also in inflammatory granulomas and stress. Here we propose the design of a modular vaccine based on outer membrane vesicles (OMV´s) of the bacterial fish pathogen *Aeromonas salmonicida* (*A. salmonicida*.). The simple preparation, the safety due to their non-replicative nature as well as the composition of natural surface exposed membrane antigens in their native confirmation are the advantages of such a vaccine design. In the present project, the innate immune response to OMV´s in comparison to bacterial stimulation was characterized using a peritoneal model for rainbow trout (*Oncorhynchus mykiss*). The distribution, recruitment and kinetics of myeloid cell populations in peritoneum, blood, spleen and head kidney were compared using lineage marker specific monoclonal antibodies. Additionally, the monocyte/macrophage cell line RTS-11 was used to characterize the mRNA profile response of phagocytes to OMVs and *A. salmonicida* bacterial particles.
First results indicate similar patterns of cellular responses in vivo either by stimulation with OMVs or with bacteria in regards to cell kinetics as well as to the induction of pro-inflammatory genes.
Next steps will include engineering of recombinant *A. salmonicida*, which produce of OMVs, presenting the immunogenic G-proteins of Viral-hemorrhagic-septicemia-virus (VHSV), Infectious-hematopoietic-necrosis-virus (IHNV) and Spring-viraemia-of-carp-virus (SVCV).Those OMV´s will be used to analyze the innate immune response against bacterial and viral pathogens in regard to induction of protective immune memory.

KEYWORDS
Aeromonas salmonicida, outer membrane vesicles, *Oncorhynchus mykiss*, innate immune response, vaccine

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O-042

IMPACT OF SEA LICE (*Caligus rogercresseyi*) INFECTION LEVELS ON SKIN TRANSCRIPTOME IN ATLANTIC SALMON (*Salmo salar*)

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ABSTRACT
Skin is a complex mucosal tissue, which is exposed to the outer environment, interacts with a plethora of environmental insults and represents the first line of defence against many pathogens. Sea lice *Lepeophtheirus salmonis* and *Caligus rogercresseyi* constitute a major threat to farmed salmonids, in both Northern and Southern hemispheres, respectively. We examined the response of skin transcriptome to different levels of infection with *C. rogercresseyi* in Atlantic salmon (*Salmo salar*). The infections were carried out on post- smolts using copepods, with a non-infected, control group. On day 14 post- infection, fish were examined for the total number of lice and grouped into low- and high-infected individuals, with 25 ± 5.3 and 59 ± 9.2 lice per fish, respectively. Skin samples (~100 mg) were collected from the area anterior to the dorsal fin and midway to the lateral line. In total, 36 samples samples –with 12 fish allocated to control, low-infected and high- infected group, accordingly- were subjected to total RNA extraction, followed by library preparation and RNA- seq (Illumina NextSeq 500 platform, single reads 150bp long, sequencing depth 20M reads per sample). Raw reads were aligned to the Atlantic salmon reference genome (GCA_000233375.4 ICSASG_v2) and assessed for differential gene expression using EdgeR. Differentially expressed genes were identified following statistical analysis many of which could be defined as immune response genes. Of interest, genes from encoding mucins were detected as being highly enriched in the infected fish compared to control, suggesting an increase in transcription of this gene family. Other genes of interest included lectins and antimicrobial peptides such as hepcidin and cathelicidin. Many cytokines were also found modulated suggesting a complex immunological response. Full gene set enrichment is being performed to define more comprehensively the immune pathways responding to the lice infection.

KEYWORDS
Atlantic salmon, transcriptomics, *Caligus rogercresseyi*, RNA-Seq, differential gene expression

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O-043

PESTICIDE DRUGS USED AGAINST SEA LICE CAUSE TRANSCRIPTOME CHANGES IN THE EARLY STAGES OF THE NON-TARGET SPECIES *Choromytilus chorus*

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ABSTRACT

Azamethiphos and deltamethrin are pesticide drugs commonly used in Chile as control treatments for sea lice outbreaks in salmon farms. However, their effect on many biological processes of non-target marine species is still unknown. Here we described transcriptome patterns of the mussel *Choromytilus chorus* associated to the effect of these drugs on the early stages of its lifecycle. Trocophore and D larvaes were submitted to continuous exposure to azamethiphos and deltamethrin drugs at 10 and 1000 ppb during 5 days. Samples were collected for RNA-sequencing and transcriptomic analyses were conducted to evaluate gene expression and annotation. Short-read sequencing were conducted in the Ilumina HiSeq platform and long-read sequencing on the Oxford's Nanopore MinION platform. Mortality was not observed in either trocophore or D larvaes exposed to both drugs. Also, fertilization was successful at 80% or more in all treatments. However, thousands of assembled contigs were differentially expressed (FC > |4|; p-value < 0.01) in RNA-seq analyses from samples exposed to drugs with respect to control. Time of exposure to drugs triggered larger expression changes with respect to control groups than different chemical concentrations. Annotation by Gene Ontology of differential expressed genes revealed that azamethiphos exposure were mostly associated to changes in genes related to general metabolic processes, while deltamethrin to binding proteins. This study contributes to the understanding of the abnormal effects at molecular level that pesticides over early stages of the mussel *C. chorus* as non-target species and can have potential impacts on the later development of this economically important species.

KEYWORDS: Non-target species, mussel, sea lice, azamethiphos, deltamethrin.

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O-044

UNDERSTANDING VIRULENCE AND PATHOGENESIS IN CONTROLLED CONDITIONS: THE CASE OF BROWN RING DISEASE CAUSING IMMUNODEPRESSION IN CLAMS

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ABSTRACT
The Brown Ring Disease (BRD) has caused high mortality rates since 1986 in the Manila clam *Venerupis philippinarum*, which was introduced in Western Europe in the 1970s. The causative agent of BRD is a Gram-Negative bacterium, *Vibrio tapetis,* which is also pathogenic to fish. Infection of clams inside the adductor muscle has been done and intra-cellular fate of *Vibrio tapetis* has been investigated inside hemocytes using Transmission Electronic Microscopy. Intracellular multiplication of *V. tapetis* inside hemocytes has been followed during three days, showing that *V. tapetis* could be able to interfere with the autophagy process. Comparative genomic analysis of 17 *Vibrio tapetis* strains allowed identifying a type IV secretion system (T4SS) only present in strains virulent to clams. To further characterize the mechanisms underlying pathogenicity in *V. tapetis* CECT4600, we developed a gene deletion approach to determine the role of different genes in infection. In particular, we created a D *virB4* null mutant by deleting the full *virB4* gene, which encodes a protein essential to the T4SS assembly and functioning. We performed *in vitro* virulence tests on the wild type and the deleted strains, and showed that the D*virB4* mutation cancelled the virulence of *V. tapetis* to clams. These results proved that our approach is appropriate to explore and understand the role of multiple genes in the *V. tapetis* pathogenicity. Our on-going experiments have the objective to further characterize the role of T4SS, as well as other genes, in the *V. tapetis* infection and the development of BRD in the Manila clam.

KEYWORDS
Vibriosis, clams, autophagy, T4SS, mutagenesis.
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O-045

INVOLVEMENT OF MYTICINS IN TISSUE INJURY IN MEDITERRANEAN MUSSELS

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ABSTRACT
Mediterranean mussels (*Mytilus galloprovincialis*) are sessile filter feeders that live in close contact with numerous marine microorganisms. As all invertebrates, they lack an adaptive immune response and how these animals are able to respond to a bacterial infection and discriminate it from their normal microbiome is difficult to understand.
The analysis of mussel hemocytes transcriptome before and after the animals being infected with *Vibrio splendidus* revealed an important reaction against a tissue injury, even without bacteria, in which the myticins, the most expressed antimicrobial peptides in mussel, appeared significantly up regulated. Functional experiments with flow cytometry confirmed these transcriptomic results, seeming that myticins would be involved in the response to a danger signal such as a simple injection in the adductor muscle.
Moreover, mussel hemocytes stimulated with myticin C (*in vitro* approach) showed a transcriptomic response basically related to cytoskeleton and contraction, as well as maintenance of tissues and cell structures integrity. All results suggest a new role of myticin C, an antimicrobial peptide traditionally related to immune response and defense against pathogens, which seems to be important after a tissue damage.

KEYWORDS
Mussel, tissue injury, myticin C, RNAseq, hemocyte

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O-046

THE SYDNEY ROCK OYSTER MICROBIOME IS INFLUENCED BY LOCAL ENVIRONMENTAL PARAMETERS AND QX DISEASE RESISTANCE

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ABSTRACT

Sydney rock oysters, (SRO: *Saccostrea glomerata*) are a native species in Australia and the most important aquaculture species in the state of New South Wales (NSW). However, production of this species has declined significantly since the mid-1970s, in part due to the impacts of mortality events associated with QX (Queensland unknown) disease. QX disease is caused by a spore-forming protozoan parasite called *Marteilia sydneyi* however, the presence of the parasite does not necessarily result in QX disease indicating the role of environmental and/or host-specific factors in disease progression. Another potential factor in QX disease is the microbiome of the SRO, however, little research has been conducted into the microbiome of this oyster species. In this study, we examined the microbiome of six families from the SRO breeding program with differing resistance to QX disease (two highly resistant, two with intermediate resistance and two susceptible) deployed in two different locations using 16S rRNA (V1 – V3 region) amplicon sequencing. The broad aim of this study was to determine the effect of local environmental parameters and disease resistance on the microbiome of the SRO. Our results show that microbiomes of SRO families significantly differed between the two deployment locations of Port Stephens and Wallis Lake (NSW), and between our two sampling points in the Austral summer and winter. Additionally, the SRO microbiome was influenced by QX disease resistance at Port Stephens at both time points with the susceptible lines significantly differing from the resistant and intermediate families. However, in Wallis Lake, the influence of host-specific QX disease resistance was not consistent over the two seasons suggesting that environmental factors can overcome the influence of host genetic factors.

KEYWORDS: Microbiome, Sydney rock oyster, QX disease resistance, selective breeding, Port Stephens, Wallis Lake

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O-047

B-GLUCAN IMMUNO-MODULATION IN COMMON CARP INTESTINE: A ROLE FOR MICROBIOTA AND ITS METABOLITES

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ABSTRACT

Dietary supplementation of fish with β-glucans has been widely associated with immunomodulation and commonly accepted as beneficial for fish health. However, to date the exact mechanisms of immunomodulation by β-glucan supplementation in fish are still largely unknown. In mammals a clear relation has been observed between high fibre diets and immunomodulation via intestinal microbiota and its metabolites. In this study, we first described the normal microbiota of common carp intestine by 16S rRNA sequencing. Based on the abundance of the genus *Bacteroides*, well known for their capacity to degrade and ferment carbohydrates, we hypothesized that common carp intestinal microbiota could ferment dietary β-glucans. Indeed, two different β-glucan preparations (curdlan and MacroGard®) were both fermented *in vitro* albeit with distinct fermentation dynamics and significant differences in production of short chain fatty acids (SCFA). MacroGard® more than curdlan lead to production of propionate, a SCFA with immunomodulatory properties. Subsequently, *in vivo* treatment effects of a single oral gavage with MacroGard® were analysed. Intestinal microbial composition seven days post-treatment showed a significant shift towards the family *Rhodocyclaceae*, including *Propionibacterium* sp, known to synthesize propionic acid by using unusual transcarboxylase enzymes. Coinciding with the shift in microbial composition, an overall immunomodulation could be observed as inhibition of expression of several pro-inflammatory genes (*il1β*, *il6, tnfα*). Based on our data, we discuss the possibility that fermentation of MacroGard® by specific bacteria part of the normal microbiota of common carp intestine can lead to a shift in microbial composition and associated production of the SFCA propionate, the increased presence of which could possibly explain (part of) β-glucan-induced immunomodulatory effects.

KEYWORDS

Microbiota, β-glucan, SCFA, *Cyprinidae*, 16S rRNA sequencing

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O-048

THE TRANSCRIPTOME REVEALS THE MOLECULAR MECHANISMS UNDERLYING HEMOCYTES DIFFERENTIATION IN THE OYSTER

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ABSTRACT
Hemocytes are considered to be the central component of internal immune defense system in many invertebrates including oysters. Morphological and functional classification demonstrate that the granulocytes rather than hyalinocytes are the main immune executor since the former exhibited stronger capacities either in phagocytosis or ROS production. However, the molecular mechanism underlying granulocytes cytogenesis is still unclear. In this study, two main cell types, granulocytes and hyalinocytes were sorted and further studied based on the flow cytometry from hemocytes of the Pacific oyster *Crassostrea gigas*. Transcriptomic analyses from four biological replications revealed the 175 of core differentially expressed genes (DEGs) were significantly high expressed in granulocytes when compared with hyalinocytes. Moreover, these core DEGs were also highly expressed specifically in hemocytes rather than other tissues. The pathway network analysis revealed that phagocytes were highly activated together with actin cytoskeleton regulation, phagosome, MAPK signaling, lysosome and others. Meanwhile, the cdc42, as one of the key hub-genes connected multiple pathways, was also confirmed to be involved in regulation of phagocytosis and ROS production by treatment of pharmacological inhibitors. Finally, the RNAi of the key transcriptional factors show that FOS are responsible for transcriptional activation of many phagocytosis and ROS production-related genes, and typical FOS-binding sites (AP-1) were also found in proximal promoter of these genes, strongly suggesting the regulatory role of FOS in the cytogenesis of granulocytes. In a word, these results provide a novel insights into gene regulatory network underlying hemocytes differentiation of oyster.

KEYWORDS
Oyster, hemocytes differentiation, granulocytes, phagocytosis, FOS

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O-049

IMMUNE RESPONSES TO BACTERIAL INFECTION IN ZEBRAFISH JUVENILES WITH DIFFERENT EARLY-LIFE INFECTIOUS HISTORIES

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ABSTRACT
Early-life adversity has an important influence on immune responses during the lifespan development. In mammals, early-life stress was related to long-term consequences on immune functions. In fish, the exposure to a brief handling stressor or xenobiotics during early-life influenced the stress sensitivity and immunity in adulthood. So, it seems that challenges of various natures (e.g. social, chemical, physical or biological) during early-life can impact or shape an individual physical and mental's health across the lifespan in fish. Bacterial infections during early life (embryonic and/or larval) stages are very common in fish, long before the attainment of immunocompetence, when the immune system is still developing. Zebrafish larvae only possess a fully developed immune system by 4-6 weeks post fertilization. During the first weeks of life, zebrafish larvae simply rely on efficient components of the innate immune system, most of which are already functional at the first day of embryogenesis. In this study, we aimed to evaluate the effects of different bacterial challenges during early development on zebrafish and on its immune system later in its life, notably for genes involved in immune responses against pathogenic infection. Thus, four histories of infection with a virulent strain of *Aeromonas salmonicida achromogenes* were tested in the first month post-hatching: control group without any infection, zebrafish exposed to an early infection at 15 days post-hatching (dph), zebrafish chronically exposed to bacteria, from 15 to 32 dph, and zebrafish exposed later at 32 dph. Then, all groups were maintained in tanks and exposed to the same pathogen at 58 dph. Fish were sampled before infection, and at 6h and 24h post-infection. The analysis of immune gene expression in early life stages of fish revealed that the age of first infection influenced the responses (level of expression and timing) of immune system including bacterial lysis, opsonization or phagocytosis (*C3a component, myeloxyperoxidase*), inflammatory processes (*il-6* and *cox 2*) and adaptive immunity (*cd4, rag 1* and *tcra*). In addition, the exposure to the pathogen in juvenile stage (58 dph) induced differential innate immune (including *c3a, lysozyme, mpo, transferrin*) and inflammatory responses (*il-6*, *cox2, tgfb*) depending on infectious history during the early life. These results suggest that the infection at larval stage, when adaptive responses are not yet effective, will influence immune pathways later in life and offer new perspectives to study the molecular modifications affecting the memory of immune system such as epigenetic mechanisms.
Immune responses to bacterial infection in zebrafish juveniles with different early-life infectious histories

KEYWORDS
Zebrafish; bacterial challenge; early infection; long-term effects; molecular analysis.
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O-050

DISTRIBUTION OF DIFFERENT POPULATIONS OF T CELLS AND B CELLS IN THE INTERBRANCHIAL LYMPHOID TISSUE (ILT) OF SALMON

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ABSTRACT
The interbrancheal lymphoid tissue of Atlantic salmon (*Salmo salar*) consists of T cells embedded in a matrix of reticulated epithelial cells. The amount of B cells is very limited. Here we use *in situ* hybridization probes to detect transcripts of different specific T and B cell genes. We show that the structure is rich in α/β T cells but less so in γ/δ T cells. In the thymus, the distribution of such cells is localized in distinct zones, but that is not the case in the ILT. The constitutionally expressed cytokine CCL19, which is important in T cell zones in mammalian lymphoid tissues and attracts naïve T cells (but also other leukocytes), is evenly distributed in the ILT but restricted to cortical regions in the thymus. IgT expressing cells in the ILT are less common that IgM-expressing cells. Control tissues included skin and head kidney. The use of *in situ* hybridization has allowed us to reveal specific anatomical features of the ILT and the thymus regarding leukocyte cell distribution. The experiments have shown that even though the ILT and the thymus share some common features, the anatomical organization of the tissues are profoundly different.

KEYWORDS
B cell; gills; ILT; T cell; thymus

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O-051

B-GLUCAN IMMUNO-MODULATION IN COMMON CARP INTESTINE: A ROLE FOR MICROBIOTA AND ITS METABOLITES

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ABSTRACT

Dietary supplementation of fish with β-glucans has been widely associated with immunomodulation and commonly accepted as beneficial for fish health. However, to date the exact mechanisms of immunomodulation by β-glucan supplementation in fish are still largely unknown. In mammals a clear relation has been observed between high fibre diets and immunomodulation via intestinal microbiota and its metabolites. In this study, we first described the normal microbiota of common carp intestine by 16S rRNA sequencing. Based on the abundance of the genus *Bacteroides*, well known for their capacity to degrade and ferment carbohydrates, we hypothesized that common carp intestinal microbiota could ferment dietary β-glucans. Indeed, two different β-glucan preparations (curdlan and MacroGard®) were both fermented *in vitro* albeit with distinct fermentation dynamics and significant differences in production of short chain fatty acids (SCFA). MacroGard® more than curdlan lead to production of propionate, a SCFA with immunomodulatory properties. Subsequently, *in vivo* treatment effects of a single oral gavage with MacroGard® were analysed. Intestinal microbial composition seven days post-treatment showed a significant shift towards the family *Rhodocyclaceae*, including *Propionibacterium* sp, known to synthesize propionic acid by using unusual transcarboxylase enzymes. Coinciding with the shift in microbial composition, an overall immunomodulation could be observed as inhibition of expression of several pro-inflammatory genes (*il1β*, *il6, tnfα*). Based on our data, we discuss the possibility that fermentation of MacroGard® by specific bacteria part of the normal microbiota of common carp intestine can lead to a shift in microbial composition and associated production of the SFCA propionate, the increased presence of which could possibly explain (part of) β-glucan-induced immunomodulatory effects.

KEYWORDS

Microbiota, β-glucan, SCFA, *Cyprinidae*, 16S rRNA sequencing

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O-052

TRANSCRIPTOME AND PROTEOME ANALYSES OF RED BLOOD CELLS FROM RAINBOW TROUT CHALLENGED WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

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ABSTRACT

Teleost red blood cells (RBCs) have gained interest due to the fact that are nucleated and possess intracellular machinery necessary to develop a response to pathogens. Several studies have lately pointed out the implication of RBCs in the immune processes against viral infections. We have recently demonstrated that rainbow trout (*Oncorhynchus mykiss*) RBCs can also mount an immune response against abortive viral hemorrhagic septicemia virus (VHSV) infection *in vitro*. In this work, rainbow trout were challenged with VHSV. After two days post-challenge, peripheral blood and head kidney tissue samples were collected. For the transcriptomic analysis, RBCs were purified from these samples using a single cell sorting technique. For the proteomic analysis, RBCs were purified by two consecutive density gradient centrifugations. Results from the transcriptomic and proteomic analyses revealed an upregulation in genes from several immune-related GO-terms categories such as type I interferon, antigen presentation, complement activation and humoral response. Clusterization of genes from RBCs of peripheral blood and head kidney indicates a complementary profile, where downregulated genes from head kidney RBCs have a higher expression in blood RBCs and vice versa. In summary, in this work we show for the first time that RBCs can develop an immune response during *in vivo* VHSV infection of rainbow trout.

KEYWORDS

Transcriptomics, proteomics, red blood cells, VHSV, complement

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O-053

FROM LAMB TO LION: UNLEASHING THE BEAST IN "VIRULENT" *Aeromonas hydrophila*

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ABSTRACT
An emerging pathotype of *Aeromonas hydrophila* (vAh) has been responsible for widespread farm losses in the US catfish industry over the last decade. While our genetic and biochemical understanding of vAh has been greatly enhanced in this time frame, our ability to reliably induce the disease in the laboratory remained limited. Utilizing established protocols for Aeromonad challenges resulted in minimal mortality and inconsistent clinical symptoms. Therefore, taking cues from observed farm conditions associated with outbreaks, we perturbed iron scavenging dynamics and catfish feeding status. Addition of a xenosiderophore, deferoxamine mesylate (DFO), to vAh cultures prior to immersion challenge significantly increased virulence in several vAh isolates but not in a non-epidemic strain. DFO addition did not impact vAh growth dynamics or perturb iron-sensitive gene pathways, but did significantly enhance hemolysis of catfish blood. Furthermore, hours between last feeding and immersion challenge (postprandial status), was observed to be a critical determinant of catfish susceptibility. Fish with a full gastrointestinal tract had significantly lower survival than those in a fasted state, and this effect was cumulative with that of DFO-enhanced vAh virulence. Utilizing our more robust challenge model, we are currently examining the practical efficacy of varying protective strategies for the industry including diet modification, vaccination, genetic selection, and modulation of the pond environment. Our latest results in this vein will be presented.

KEYWORDS
Host-pathogen; Aeromonas; catfish; iron; siderophore
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O-054

STOMACH METABOLIC ALTERATIONS IN RESPONSE TO AHPND INFECTION IN SHRIMP

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ABSTRACT

Acute hepatopancreatic necrosis disease (AHPND), previously termed early mortality syndrome, is an emerging shrimp disease with very serious impacts on Asian shrimp aquaculture. This disease is caused by pathogenic bacteria *Vibrio parahaemolyticus* (Vp) containing a unique virulence plasmid (pVA), with genes encoding a binary toxin PirABVp. Molecular pathogenesis of this emerging disease and host responses against AHPND remain unclear. To further explore AHPND pathogenesis, a systems biology approach was used to identify the significant pathway[s] in transcriptome and metabolome of AHPND-infected shrimp stomach. Using UHPLC-QTOF-MS-based metabolomics profiling, 503 and 634 differentially expressed metabolites were selected from positive and negative ion modes. These metabolites were combined with our in-house transcriptome database to obtain global host responses in stomachs of AHPND-infected shrimp. With this strategy, it was determined that several lipid metabolism related pathways in shrimp stomachs were dysregulated during AHPND infection. A gene-to-gene correlation network was created to identify candidate genes, with gene expression subsequently confirmed with real-time PCR. Cytosolic phospholipase A2 (cPLA2) and JHE-like carboxylesterase (JHE-L CE) were significantly expressed in stomachs of AHPND-infected shrimp. These findings are new knowledge regarding AHPND pathogenesis and will contribute to development of an evidence-based biosecurity approach for shrimp aquaculture industry.

KEYWORDS

AHPND, metabolomics, lipid metabolism, Cytosolic phospholipase A2, JHE-like carboxyesterase

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O-055

CHARACTERIZATION AND DEVELOPMENT OF FOCAL RED AND MELANISED CHANGES IN THE ATLANTIC SALMON

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ABSTRACT

The occurrence of melanised changes in the muscle fillets in the Norwegian salmon industry is approximately 20% and leads to quality reduction and severe economic losses. The condition is common for Atlantic salmon farming in general, but prevalence are not available outside Norway. Here, we investigated the occurrence of focal red and melanised changes in a regular field production net-pen population, throughout the saltwater production period. We found that the prevalence of focal acute red changes was stable throughout the production period and that these changes develop into focal melanised changes over time. The presence of bacteria and virus in focal red changes was addressed but could not explain the acute manifestations, however, accumulations of lipids was a common finding. Lipid depositions were located within degenerative muscle cells. Infection with *Piscine orthoreovirus* 1 (PRV-1), which is ubiquitous in sea farmed Atlantic salmon, appeared in the population at Week 23 post sea transfer. Chronic melanised changes appeared after the identification of PRV-1, and cells with replicating virus was always present in the chronic melanised changes that were characterized as granulomatous inflammation. We further addressed the inflammatory responses by targeting different cell markers.

KEYWORDS

Granuloma; inflammation; lipids: melano-macrophage; PRV

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O-056

SEASONAL ADAPTATION OF THE IMMUNE SYSTEM RELATED TO REPRODUCTIVE CHANGES IN THE EUROPEAN SEA BASS: AN INTEGRATIVE AND SYSTEMIC VIEW

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ABSTRACT

During winter-season, European sea bass, Dicentrarchus labrax, experiences low temperature whilst, at the same time, it invests into gametogenesis. Such abiotic and biotic factors are well known to affect the energy budget and to modulate energydemanding functions, amongst them the immune system. The mechanisms of immunomodulation related to reproductive investment and environmental adaptation are, however, not well understood. Therefore, we sampled primary and secondary lymphoid organs including thymus, head-kidney, spleen and gills from juveniles and mature/prepubescent sea bass in winter and in summer. The proportion of DLT15+ and DLIg3+ lymphocytes as well as the capacity for phagocytosis and oxidative burst was measured by flow cytometry in isolated leucocytes. DLT15 and DLIg3 are monoclonal antibodies specific for sea bass pan-T cell marker and IgM, respectively. To evaluate the differences in the energetic input between season and ages, biometric parameters including the hepatosomatic, the splenosomatic, the gonadosomatic and the peritoneal adiposomatic indices were recorded. The biometric measurements (length, gonadosomatic and adiposomatic indices) confirmed that, from summer to winter, juveniles mainly invest in body growth while mature/prepubescent sea bass dedicate their energy into reproduction.

In both groups, the winter-season altered the T cell-proportion in the thymus, the gills and the spleen. Contrariwise, the proportion of IgM+ lymphocytes increased in the spleen of juveniles and mature/prepubescent sea bass as well as in the thymus and the head-kidney of juveniles only. In agreement, the leucocyte number per head-kidney was drastically increased in juvenile and mature/prepubescent specimen, whereas the number of isolated thymocytes per thymus, dropped only in the mature/prepubescent fish. These results are in agreement with previous findings, describing thymus involution in other fish species as well as increased plasmatic natural antibodyconcentrations in Sea bass during winter-season. In summary, it is proposed that sea bass inhibits the adaptive immune system (T celldevelopment and -peripheral fraction) in favor of the innate component and more specifically the humoral immunity represented by natural antibodies in order to adapt to winter-conditions and the reproductive investment during winter. Ongoing work aims to analyze (1) the plasmatic steroid hormone-levels to characterize the reproductive states and explain gender differences, (2) the immunohistochemical changes in the immune

organs and (3) gene expression for T and B cell-differentiation.

KEYWORDS
B cell, T cell, thymus, head-kidney, eco-immunology
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O-057

NODAVIRUS MODULATES IMMUNE-RELEVANT PROTEINS IN EUROPEAN SEA BASS

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ABSTRACT

European sea bass (*Dicentrarchus labrax*) is the most important fish species in Spanish aquaculture in terms of biomass (Tm) production. One of the biggest problems facing its farming is its great susceptibility to nodavirus infection, which causes mortality rates up to 100% in larvae and juveniles. The knowledge of immune responses triggered upon nodavirus infection in European sea bass specimens and their regulatory mechanisms is mandatory to maintain the production of this specie. Nodavirus up-regulates the transcription of genes coding for antimicrobial peptides, cellular markers of T and B lymphocytes and pro-inflammatory cytokines whether inhibits those genes related to the interferon type I pathway in the brain. At this point, an insufficient antiviral response at transcriptional level is shown leading to develop the viral disease. In this work, and due to the lack of tools to characterize the leucocytes using specific cell populations markers, we have produced polyclonal antibodies specific to European sea bass antimicrobial peptides (NK-lysin and dicentracin), interferon gamma and perforin to quantify them through larval development and study their regulation in control and nodavirus-infected juveniles. Our results show basal levels of these proteins during the entire larval development from eggs up to 69 days post-fertilization, increasing at two different time points in the case of several proteins. After nodavirus infection, the quantification of most proteins decreased instead of increasing as expected upon activation of an immune response. These data suggest a post-translational modulation of these proteins by the virus since the enhancement of antimicrobial activities was previously demonstrated. Taking into account the lack of preventive or palliative solutions to nodavirus infections, further investigations should be developed to understand how nodavirus evades the immune response to spread.

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KEYWORDS
Nodavirus, protein, polyclonal antibodies, immune response, *Dicentrarchus labrax*

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O-058

MUCOSAL IMMUNOGLOBULIN IGT PLAYS A KEY ROLE IN THE ORAL IMMUNITY OF TELEOSTS

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ABSTRACT
The oral gustatory organ of vertebrates is crucial to obtain energy for survival and reproduction, and it is simultaneously exposed to pathogenic organisms. Thus, oral-associated lymphoid tissue (OALT) is considered the first line of immune and by far, it has only been described in terrestrial animals. Since teleost fish represent the most ancient bony vertebrates containing the oral mucosa, we hypothesized that the relationship between the oral gustatory surface and mucosal immunity represents an ancient association. Supporting this hypothesis, we show for the first time that OALT is present in teleost fish and is similar to other mucosa-associated lymphoid tissues (MALTs). Moreover, we discover that the majority of bacterial microbiota in the oral mucosa is coated with IgT and, to a much lesser degree with IgM and IgD. In addition, following parasitic infection, significant specific-IgT immune responses were observed in the oral mucus, while IgM responses were almost exclusively detected in the serum. In contrast, parasite-specific IgD was absent both in oral mucus and serum. Importantly, we detect significant IgT+ B cell proliferative responses in the oral mucosa but not in head kidney and spleen of fish that survived parasitic infection, providing the first demonstration that IgT is the main immunoglobulin player in oral mucosal immunity and that IgT responses are probably induced locally in the oral mucosa. More critically, we reveal that the teleost oral mucosa is a novel and effective site of immunization for the control of aquatic parasitic infection. Overall, our findings further broaden the understanding of oral immunity in not only terrestrial animals but also in early vertebrates.

KEYWORDS
Oral immunity, IgT, B cells, Mucosal immune, Rainbow trout (*Oncorhynchus mykiss*)

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O-059

17α-ETHINYLESTRADIOL OR TAMOXIFEN ALTERS THE HUMORAL INNATE IMMUNE FUNCTION IN MALE GILTHEAD SEABREAM

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ABSTRACT

The presence of pharmacological compounds in the marine water have increased the concern about their unpredicted effects in aquatic organisms. 17α-ethinylestradiol (EE2), a potent estrogenic compound, is widely used in oral contraceptive pills treatments and hormonal therapies. Tamoxifen (Tmx), an antagonist or agonist of the estrogen receptor alpha depending on the cell types, is commonly used in breast cancer therapies. Both drugs are present in aquatic environments. The gilthead seabream (*Sparus aurata*) is one of most important species in Mediterranean aquaculture and the effects of these compounds in its physiology are of especial relevance. It is demonstrated that cellular and adaptive humoral immune responses are altered by both compounds in a manner than depends on the age and the reproductive stage of fish. The innate immune function in fish is the first line of defense against pathogens and it is of great importance in poikilothermic animals. In this work we have studied the effect on different humoral innate immune responses of gilthead seabream upon dietary exposure to EE2 or Tmx at different ages and reproductive stages. Our results show that both compounds modulate the humoral innate immune response and that the exposure animals needed different times to recover control values upon the cease of the treatment depending on fish age, the reproductive stage and the length of the treatments.

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KEYWORDS

17α-ehinylestradiol, tamoxifen, innate immune system, *Sparus aurata*, males

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O-060

TRYPANOSOME-HOST INTERACTION REVEALED THROUGH THE ZEBRAFISH LOOKING GLASS

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ABSTRACT

Trypanosoma carassii is an extracellular blood parasite of cyprinid fish phylogenetically closely related to *Trypanosoma brucei*, the causative agent of the sleeping sickness disease in humans and livestock. Motility is crucial for trypanosome pathogenicity, but real-time visualization of parasite movement *in vivo*, in the natural host environment, has not been reported thus far. In this study, we report the establishment of *T. carassii* infection in zebrafish (*Danio rerio*), which allowed us, for the first time in a vertebrate host, to characterize in details the movement of trypanosomes *in vivo*. By combining the transparency of zebrafish larvae with the availability of several transgenic lines marking macrophages, neutrophils, cytokine-expressing leukocytes and endothelial cells, we were able to study in real-time: 1) parasite movement *in vivo*; 2) the kinetics of innate immune responses; and 3) parasite interaction with host (immune) cells. Our results indicate that during *T. carassii* infection of young zebrafish a differential macrophages and neutrophils response is observed. Macrophages responded more prominently than neutrophils by proliferating, and were massively recruited to blood vessels. Macrophages also exhibited heterogeneous morphologies and a strong pro-inflammatory profile. In fact, they were strongly positive for Tnfα and Il-1β and had a morphology characteristic of foamy macrophages. Large foamy macrophages accumulated in the portal vein of highly infected individuals, and were strongly positive for lipid staining, which revealed the abundance of lipid bodies in their cytoplasm. Finally, with respect to parasite movement and interaction with the host, using high-speed videography, we were able to capture novel mechanisms of parasite-host cell interaction, and to follow the onset of anemia, vasodilation and extravasation typical of trypanosome infections. Altogether, this is the first report of an *in vivo* trypanosome infection model in a natural vertebrate host describing both, the pathogen behavior and the host response. Considering that trypanosomes can infect all vertebrates, including humans, livestock and fish, our infection model is a relevant complementary tool to gain more insights in the underlying mechanisms of trypanosome infections.

KEYWORDS

Trypanosome, TNF-α, IL-1B, foamy macrophages, *in vivo*

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O-061

IS PALLIAL MUCUS INVOLVED IN OYSTER DEFENSE AGAINST THE PARASITE *Bonamia ostreae*?

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ABSTRACT

Bonamia ostreae is an intrahemocyte parasite responsible for severe mortalities in the flat oyster *Ostrea edulis* since 70´s. The Pacific oyster *Crassostrea gigas* is considered resistant to the disease and seems to develop mechanisms to avoid the infection. Most of studies carried out on invertebrate immune system focus on the roles of hemolymph, although mucus could also act as a barrier against pathogens. In this study, the effect of mucus from both oyster species, *O. edulis* and *C. gigas,* on *B. ostreae* was investigated *in vitro* using flow cytometry. Results showed an increase in esterase activities and mortality rate of parasites exposed to mucus from both oyster species. Also, the mucus seems to have an effect on the internalization of the parasite inside the hemocytes of *O. edulis*, while parasites non-exposed to mucus were highly internalized, those with mucus exposition present a lower rate of internalization, suggesting some mechanisms to neutralize the parasite activities. In order to better understand the potential role of mucus in the defense of the oyster against parasite *B. ostreae*, liquid chromatography and tandem mass spectrometry were used to describe and compare protein composition between mucus from both oyster species. Whatever the oyster species is, pallial mucus displays a great variety of proteins. More than 1800 proteins were identified in *C. gigas* mucus while 767 proteins were identified in *O. edulis* mucus. This huge difference is derived from the availability of the protein database used, *C. gigas* proteome was used to identified the proteins and some mismatch in *O. edulis* could be derived from the lack of a proper genome reference. However, *C. gigas* mucus showed more proteins related to antiviral replication such as ferritin and Ras-related protein Rab7a, as well as, antioxidant proteins such as superoxide dismutase; which is 100 times more expressed in *C. gigas* than in *O edulis*. Conversely, more protease activity proteins such as Cethepsin β and Z and apoptotic related proteins like Ubiquitin conjugatin protein enzyme E2-N were identified in mucus from the flat oyster. Our results suggest an adaptation of oysters to develop a specific response against their specific pathogens; while *C. gigas* is more susceptible to herpes virus (OsHV-1) and bacterial infection (*Vibrio aesturianus*); *O. edulis* oyster is more susceptible to protozoan parasites (*B. ostreae* and *Marteilia refringens*). These results also provide new insights for further investigations in the immune response in oysters.

KEYWORDS
Bonamia ostreae, oysters, flow cytometry, immune response, proteome.

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O-062

VACCINATION AND IMMUNE RESPONSE OF THE PITUITARY IN FISH

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ABSTRACT

Although it is known that pituitary hormones can have a direct or indirect influence stimulating or suppressing the immune responses, whether there is a local immune response in this tissue or what is the effect of the immune stimulus on the pituitary function in fish has not received specific attention. In order to understand the immuno-endocrine interaction at the pituitary level, particularly the Hypothalamus-Pituitary-Interrenal axis, different experiments were carried out in rainbow trout and gilthead seabream using both *in vitro* and an *in vivo* approaches. Pituitaries of rainbow trout *Oncorhynchus mykiss* were cultured *in vitro*, incubated for 3h with *Vibrio anguillarum* bacterin, bacterin plus CRH, cortisol, human recombinant IL1β, or spleen medium, and then genes involved in pro-inflammation (*il1β, il8, tnfα1, ifnγ*), anti-inflammation (*tgfβ1b, il10*), or innate immune modulation (*mhcIIa, c3, mif*) were tested. Data showed that, incubation with bacterin alone and bacterin plus recombinant IL1β or CRH, as well as medium from bacterin-treated spleen caused significant up-regulation of pro-inflammatory genes, while down-regulated the anti-inflammatory gene *tgfβ1b*. Besides, recombinant IL1β plus bacterin or alone caused raise of *mhcIIa* and *tnfa*, respectively. A second experiment assessed the response of seabream vaccinated by means of an injection with *Lactococcus garveiae* and immune gene modulation was determined after 1h, 6h and 24 hours looking at the expression of the proinflammatory genes *il1β*, *tnfa* and *cox2,* the anti-inflammatory *genes tgfβ1b, il10*, and the innate genes *lys* and *c3* corresponding to lysozyme and complement proteins. The immune genes *il1β*, *cox2* and *lys*, showed a strong expression in the pituitary tissue after injection vaccination, notably *il1β* which showed more than 10 fold raise, thus indicating both a high sensitivity to the vaccine and the onset of a robust immune response in the pituitary at *in vivo* level. The overall results indicate that pituitary shows a relevant local immune gene equipment, and also the potential of fish pituitary to develop both innate and adaptive immune responses.

KEYWORDS

Vaccination, pituitary, trout, seabream, immune-response

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O-063

THE EVOLUTIONARY PUZZLE OF IgT GENES IN ANTARCTIC FISHES

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ABSTRACT
The Perciform suborder Notothenioidei represents the major component of the Antarctic fish fauna, comprising five Antarctic families, Channichthydae, Bathydraconidae, Artedidraconidae, Nototheniidae, and Harpagiferidae, and three non-Antarctic families, Bovichtidae, Pseudaphritidae, and Eleginopidae. Notothenioids have evolved a variety of peculiar anatomical, physiological and biochemical features to adapt to the extremely cold Antarctic environment, providing an extraordinary model system to identify gene changes and investigate their importance for adaptive evolution. We have previously isolated and characterized *IgT* heavy chain constant region gene of the Antarctic teleost *Trematomus bernacchii* (family Nototheniidae), discovering that *T. bernacchii* IgT lack almost the entire heavy chain second constant domain, retaining only a few of amino acid residues. By analyzing cDNA sequences encoding IgT heavy chain three differently sized IgT transcript variants were identified, named Long, Short, and Shortest, 51-bp, 33-bp, 42-bp long, respectively. The aim of the present study is to provide a framework for understanding the loss of the CH2 domain through the notothenioid phylogeny. To this end, we isolated and characterized *IgT* genes from other species belonging to families Nototheniidae, Bathydraconidae and Artedidraconidae. In all cases the remnant CH2 exon preserved the donor and acceptor splicing sites to be correctly spliced into the mature transcript, giving rise to different cDNA variants: 24-51 bp long (8-17 aa) according to the species analyzed. Moreover, one representative each of the two non-Antarctic families was included in our studies for comparison: *Eleginops maclovinus* (family Eleginopidae), and *Bovichtus diacanthus,* (family Bovichtidae). Both diverged early from the main notothenioid lineage, before a severe decrease in water temperature and climatic and geographic isolation of Antarctica. A comparative analysis at genomic level has highlighted that the remnant CH2 exon is shared by all Antarctic fish families analyzed in the present work. Amazingly, the loss of most CH2 is shared also by *E. maclovinus* but not by *B. diacanthus.* These results may help shed light on the evolutionary processes that underlie the origins of such gene modifications.

KEYWORDS
IgT, Antarctic teleost, evolution, exon remnant, genome modifications

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O-064

HEAD KIDNEY- AND TRUNK KIDNEY-DERIVED MACROPHAGES DIFFERENTIALLY RESPOND TO STRESS AND CORISOL.

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ABSTRACT
In teleosts, hematopoiesis, including myelopoiesis, is maintained in the head (HK) and trunk kidney (TK). Moreover, HK, but not TK, functions as endocrine organ where cortisol is produced. Therefore, it is postulated that macrophages from both sources differentiate in different hormonal microenvironment where HK-born macrophages are under direct paracrine action of cortisol. Interestingly, in mammals cortisol has been described as one of the factors inducing alternative anti-inflammatory M2 polarization of macrophages.
For example, in RAW 264.7 macrophages it decreased gene expression of pro-inflammatory mediators (e.g. IL-1b and inos). Here we aim to compare effect of stress (in vivo) and cortisol (in vitro) on the polarization of carp macrophages derived from HK and TK. Carp macrophages from both sources were separated and in vitro treated (6 h) with cortisol (CORT, 1 μM), lipopolysaccharide (LPS, 30 μg/ml) or with CORT+LPS. In vivo fish were stressed (restraint stress for 24h) or left undisturbed. Macrophages from both animal groups were isolated and ex vivo treated with LPS (6h). Gene expression of several markers of classical M1 (iNOS, IL-1b, IL-12p35, CXCL8 and CXCb) and alternative M2 (arginase, IL-10, MMP-9) macrophage polarization and expression of genes encoding cortisol receptors (GR1-2) were measured. We found that CORT in vitro in LPS-treated HK and TK macrophages down-regulated gene expression of M1 markers: il-1b, cxcl8 and cxcb1 while in TK-derived macrophages CORT induced down-regulation of the gene expression of both M1 (il-12p35) and M2 (il-10) markers and both gr genes. Upon stress, freshly isolated HK macrophages had higher gene expression of M2 markers (arginase 2, IL-10 and MMP-9) than cells from control fish. Interestingly, in TK macrophages from stressed animals, next to up-regulation of IL-10 and MMP-9 genes, stress induced down-regulation of IL-1b and CXC chemokines.
Moreover, LPS-treated HK macrophages from stressed fish down-regulated il-12p35, cxcl8_l2 and cxcb1 gene expression and up-regulated gr2 expression whereas at the same conditions TK-macrophages upregulated gene expression of CXC chemokines and down-regulated expression of arginase 2. All together our data suggest that, however in fish macrophages cortisol and stress induce alternative M2 polarization this can be differentially manifested in HK- and TK-derived cells as in HK macrophages stress up-regulates M2 markers while in TK cells it up-regulates M2 markers and at the same time down-regulates M1 markers.
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KEYWORDS
macrophage polarization, stress, cortisol, carp
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O-065

DETECTION OF INTERLEUKIN (IL)-22 PROTEIN EXPRESSION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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ABSTRACT
IL-22 is a critical cytokine which is involved in modulating tissue responses during inflammation, and is produced mainly by T cells and innate leucocytes. In mammals, IL-22 is a key component in mucosal defences, tissue repair, epithelial cell survival and proliferation. In teleosts, IL-22 has been cloned and studied in several species, and the transcript is highly expressed in mucosal tissues and induced by pathogen associated molecular patterns (PAMPs), suggesting IL-22 also functions as an important component of the innate immune response in fish. To investigate these immune responses further, we have validated and characterised two monoclonal antibodies (mAbs) which were raised against two different peptide immunogens of salmonid IL-22. Our results showed that both mAbs specifically react to their own peptide immunogens and recombinant IL-22, and are able to detect the induction of native protein expression after stimulation. In flow cytometry, an increase in IL-22 positive cells was detected after stimulation *in vitro* with cytokines and PAMPs and *in vivo* after bacterial challenge. The Immunohistochemistry results showed that IL-22 is highly upregulated in the gills after challenge, both in cells within the gill filaments and in the interbranchial lymphoid tissue (ILT). Such results suggest IL-22 may have a role in triggering local antimicrobial defences in fish that may facilitate efficient microbial clearance. Hence monitoring IL-22 producing cells/protein secretion may provide an alternative mean to assess the effectiveness of mucosal vaccines.

KEYWORDS
Rainbow trout, cytokine, IL-22, protein expression, mucosal immunity

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O-066

STRUCTURE OF GRASS CARP INTERLEUKIN-2 PROVIDES INSIGHTS INTO THE EVOLUTION OF FOUR α-HELICAL CYTOKINE FAMILY

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ABSTRACT
Interleukin (IL) -2 is a member of four α-helical cytokine familywhich also comprises IL-4, IL-7, IL-9, IL-15 and IL-21. It is primarily expressed in activated CD4+ and CD8+ lymphocytes and plays a crucial role in mediating adaptive immune response. In this study, the grass carp (*Ctenopharyngodon idella*) IL-2 (*Ci*IL-2) was sequenced and its crystal structure determined. The open reading frameof the *Ci*IL-2 gene is 426 bp, that translates into a protein of 142 amino acids, with a predicted signal peptideof 20 aa.Analysis of the crystal structure revealed that the *Ci*IL-2 displayedaclassic cytokine structure consisting offour helicalbundles.Compared with the human counterpart, the *Ci*IL-2 has a remarkably straight second helixwitha significant conformational change in the region for receptor binding. Besides, the key hydrophobic amino acids whichinteract with the receptorsin mammalsare not conservedin *Ci*IL-2. The *Ci*IL-2 is predominantly expressed in lymphocyte-rich tissues such as spleen, kidney and thymus and is able to enhance the proliferation of primary leucocytesand the expression of STAT5 and interferon gamma. Our results suggestthat IL-2 could haveundergone considerable structuralchanges in order to facilitate interactionwithits receptorsduring evolution.

KEYWORDS
Grass carp (*Ctenopharyngodon idella*), fish, interleukin-2, crystal structure, evolution

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O-067

IMMUNOLOGICAL EFFECTS OF FUNCTIONAL FEEDS ON *Penaeus monodon* NATURALLY INFECTED WITH GILL-ASSOCIATED VIRUS

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ABSTRACT
Functional feeds are becoming increasingly common to help prevent and control disease losses in marine shrimp farming. Functional feeds contain additional compounds beyond the basic nutritional requirements of the animal that result in improved health and/or growth. Common additives include probiotics, prebiotics, immunostimulants, vitamins and nucleotides. In this study we assessed three functional feeds containing either B-glucan, poly-hydroxybutyrate or a marine microbial floc ingredient on their potential immunostimulatory effect on *Penaeus monodon* with pre-existing gill-associated virus (GAV) infections. Groups of *P. monodon* (mean weight of 14 g) were fed one of the functional feeds or a basal diet for two weeks. Pre-existing GAV infection loads were determined by collecting pleopod tissue from each individual on Day 0 and using RT-qPCR to quantify GAV titre. Prevalence of pre-existing GAV infections was 83% with a mean infection load of 1.25 . 106 ± 3.12 . 105 GAV copies µg-1 TNA. After 14 days of feeding the experimental diets, eight shrimp per diet were sampled and their GAV infection load was quantified to determine the relative change. GAV infection loads increased over the 14 days, however, shrimp fed the three functional feeds, B-glucan, PHB or microbial floc, on average had 10-fold lower GAV infection compared to shrimp fed the basal diet. Although the functional feeds did not clear pre-existing infections, the results suggests they may have increased the immune capacity of shrimp to better control GAV proliferation compared with the standard diet. The impact of these functional feeds was further explored by measuring several immune parameters from haemolymph samples collected from the same eight prawns that GAV titre was quantified, including total haemocyte counts, phenoloxidase activity and the production of reactive oxidative species and antioxidants.

KEYWORDS
Functional feeds, immunostimulant, GAV, immune response, shrimp

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O-068

ANTIBODY REPERTOIRE AND KINETICS IN ATLANTIC SALMON FOLLOWING VACCINATION AND CHALLENGE WITH SALMONID ALPHA VIRUS

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ABSTRACT
Immunoglobulin M (IgM) is important in protection against disease in Atlantic salmon (*Salmo salar*). For many diseases, specific antibody response may be good despite poor disease protection, and vice versa. Non-specific antibodies (NAB), broadly cross-reactive antibodies with low affinity, are typically abundant in the blood of Atlantic salmon, but their role in disease protection is poorly understood.
In this study, we have examined the antibody repertoire and the immune gene expression of Atlantic salmon following vaccination and experimental infection. Parr was vaccinated with an commercial multivalent vaccine that included inactivated Salmonid alpha virus (SAV), and three weeks after vaccination the fish was transferred to sea water and infected with SAV subtype 3 in a shedder-cohabitant model. Blood plasma and samples from lymphoid tissue and heart were collected at several time-points pre -and post-vaccination and post-infection.
Multiplex antibody assays of blood plasma from vaccinated fish showed an increase of specific antibodies to antigenic components of the vaccine after 6 - 9 weeks. In parallel, titers of non-specific antibodies increased in plasma of vaccinated fish, showing an earlier onset of increase than specific antibodies. The presence of non-specific antibodies is detected by the recognition of a synthetic hapten-carrier complex. In contrast to vaccinated fish, titers of non-specific antibodies in plasma of control (saline-injected) fish first increased after challenge with SAV. Based on these findings, high-throughput immunoglobulin sequencing (IgSeq) of the variable (antigen-binding) region of the B cell antibody receptor will be performed. With this technology, we can study the presence of shared (present in multiple individuals) and unique (present only in one individual) B-cell clonotypes in vaccinated and control fish before and after challenge. Results from microarray transcriptome analysis and RT-qPCR of selected immune genes will also be presented.
This work was funded by the Research Council of Norway, grant 267644: «*IMCOM: Development of tools for assessment of the immune competence of Atlantic salmon smolts and growers*».

KEYWORDS
Vaccination, Atlantic salmon, Salmonid alpha virus, Antibody-repertoire

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O-069

CHARACTERIZATION OF TEN CCL20-LIKE CC CHEMOKINES IN RAINBOW TROUT (*Oncorhynchus mykiss*): SEQUENCE AND EXPRESSION ANALYSIS

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ABSTRACT
Mammalian CCL20, or macrophage inflammatory protein-3α, can function as a homeostatic and inflammatory chemokine. In relation to the latter, it is responsible for the chemoattraction of lymphocytes and dendritic cells to mucosal immune sites under inflammatory and pathological conditions. CK1, CK8A and CK8B are rainbow trout (*Oncorhynchus mykiss*) CC chemokines that were reported previously to be phylogenetically related to mammalian CCL20. In the current study, an additional seven CCL20-like paralogs in rainbow trout are reported, that are divided into three subgroups (by phylogenetic tree, homology and synteny analysis) in agreement with past studies in fish. They have been designated here as: CCL20L1a (also referred to as CK1), CCL20L1b1, CCL20L1b2, CCL20L2a (CK8A), CCL20L2b (CK8B), CCL20L3a, CCL20L3b1, CCL20L3b2, CCL20L3b3 and CCL20L3b4. Like mammalian CCL20, rainbow trout CCL20-like molecules possess a high positive net charge with a pI of 9.34-10.16, that is reported to be important for antimicrobial activity. Rainbow trout CCL20-like paralogs are differentially expressed and in general highly expressed in mucosal tissues, such as gills, intestine and thymus. The expression levels of rainbow trout CCL20-like paralogs are increased during development and following PAMP/cytokine stimulation. For example, in RTS-11 cells CCL20L3b1 and CCL20L3b2 are highly up-regulated by LPS, poly I:C, recombinant(r) IFNa and rIL-1β. Trout CCL20-like paralogs are also increased after *Yersinia ruckeri* infection or poly I:C stimulation *in vivo*, with CCL20L3b1 and CCL20L3b2 again highly up-regulated. Overall, this is the first report of the complete CCL20 chemokine subfamily in rainbow trout, and the analysis of their expression and modulation *in vitro* and *in vivo*. These results suggest that teleosts possess divergent CCL20-like molecules that may have important roles in mucosal immunity.

KEYWORDS
Rainbow trout, CCL20-like chemokine, characterisation, expression, mucosal immune response

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O-070

TEMPERATURE DRIVES THE IMMUNE RESPOSE IN ATLANTIC SALMON INFECTED WITH SEA LICE: NOVEL INSIGHTS THROUGH TRANSCRIPTOME SEQUENCING ANALYSES

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ABSTRACT
The sea louse Caligus rogercresseyi is a copepod species responsible for the most relevant parasitic infections in the Chilean salmon industry. This ectoparasite causes immunosuppression and stressful conditions in farmed fishes, which led to the activation of key elements of host's immune system. However, how these host-parasite interactions are being impacted by environmental conditions such as temperature variations is still poorly understood. The aim of this study was to describe the anti-parasitic immune response of the Atlantic salmon (*Salmo salar*) against C. rogercresseyi in contrasting temperature conditions. Fish were subjected to in vivo challenge against sea lice at two temperature regimes (8 and 16º C) with 35 copepodid/fish during 25 days. Samples were collected from hosts and parasites for transcriptome sequencing using Illumina HiSeq platform. Parasitic burden was highly divergent in the two temperaturas analyzed, being the double at 16ºC (38.9% of the initial parasitic load versus 19.3% at 8ºC).
Furthermore, many coding genes, immune-system pathways and long non-coding RNAs (lncRNAs) were significantly up-regulated in infected fishes at the highest temperature. Meanwhile, highly-relevant biological processes were also up-regulated in the sea lice at the highest temperature, such as genes related to the secretome system and homeostasis. This study contributes with novel knowledge regarding how temperature changes, that normally take place in salmon farms, could dramatically change key molecular elements involved in the hostparasite interactions between salmon and sea lice. The discovery of novel lncRNAs involved in these interactions are also discussed.

KEYWORDS
Temperature changes, Atlantic salmon, *Caligus rogercresseyi*, immune response.

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O-071

EMBRYONIC INCUBATION TEMPERATURE HAS A LONG-TERM EFFECT ON THE SPLEEN IMMUNE TRANSCRIPTOME AND ITS RESPONSE TO LIPOPOLYSACCHARIDE IN ADULT ZEBRAFISH (*Danio rerio*)

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ABSTRACT
Temperature has a profound effect on the immune system of fish. However, little is known about the effect of temperature during early embryonic development on the immune system of adult fish. We incubated zebrafish (*Danio rerio*) embryos at low (24 °C), high (32 °C), and reference temperature (28 °C) until hatching (3-5 days post-fertilization, dpf). Afterwards, all the three groups were maintained at the reference temperature until adulthood (100 dpf). At 12 h post intraperitoneal injection with lipopolysaccharide, spleens were sampled and RNA was extracted. RNA-seq was performed and the immune transcriptomes were compared between the temperature groups, as well as between LPS-challenged and control fish injected with phosphate buffered saline.
Both low- and high embryonic incubation temperatures resulted in decreased expression of some immune transcripts related to cytokines (*tnfa*, *cxcl8a*, *ccl20a.3*), neutrophil (*serpinb1l2*, *ncf1*, *ncf4*) and T cell functions (*sema4ab*, *crtam*, *alcama*) in the adult fish spleen. In addition, high incubation temperature also resulted in lower transcript levels of genes involved in neutrophil and respiratory burst (*cebp1*, *lsp1*, *cyba*), endocytosis (*rab5ab*, *rab7*, *pikfyve*), and lysosomal activity (*atp6ap1b*, *atp6ap2*, *atp6v1h*). In the same temperature group, the expression of various immunoglobulins (*rag1*, *ciita*, *cd74a*) and complement components (*c1qa*, *c1qb*, *c1qc*) was up-regulated.
Numerous immune transcripts, including antibacterial factors, and those involved in endocytosis, lysosome formation, respiratory burst, and inflammatory signaling were up-regulated with LPS challenge in fish from the low incubation temperature group. In contrast, fish from the high temperature group showed a limited immune response to LPS. In fish from the reference temperature group, expression of diverse apolipoprotein transcripts was up-regulated, while the level of cytokine transcripts was decreased.
Taken together, our data demonstrate that early developmental temperature has a long-term effect on the spleen immune transcriptome of adult zebrafish. This is relevant to understand the molecular basis of the temperature-induced immune developmental plasticity in fish and is particularly relevant in the context of climate change.

KEYWORDS
Temperature, immune system, transcriptome, LPS, zebrafish

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O-072

TURBOT (*Scophthalmus maximus*) NK-LYSIN INDUCES PROTECTION AGAINST THE PATHOGENIC PARASITE PHILASTERIDES DICENTRARCHI VIA MEMBRANE DISRUPTION

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ABSTRACT

P. dicentrarchi is one of the most threatening pathogens for turbot aquaculture. This protozoan ciliate is a causative agent of scuticociliatosis, which is a disease with important economic consequences for the sector. Neither vaccines nor therapeutic treatments are commercially available to combat this infection. Numerous antimicrobial peptides (AMPs) have demonstrated broad-spectrum activity against bacteria, viruses, fungi, parasites and even tumor cells; an example is Nk-lysin (Nkl), which is an AMP belonging to the saposin-like protein (SAPLIP) family with an ability to interact with biological membranes. Following the recent characterization of turbot Nkl, an expression plasmid encoding Nkl was constructed and an anti-Nkl polyclonal antibody was successfully tested. Using these tools, we demonstrated that although infection did not clearly affect nkl mRNA expression, it induced changes at the protein level. Turbot Nkl had the ability to inhibit proliferation of the *P. dicentrarchi* parasite both *in vivo* and *in vitro*. Moreover, a shortened peptide containing the active core of turbot Nkl (Nkl71-100) was synthesized and showed high antiparasitic activity with a direct effect on parasite viability that probably occurred via membrane disruption. Therefore, the *nkl* gene may be a good candidate for genetic breeding selection of fish, and either the encoded peptide or its shortened analog is a promising antiparasitic treatment in aquaculture.

KEYWORDS

Philasterides dicentrarchi, turbot, Nk-lysin, antimicrobial peptide, antiparasitic.

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O-073

LINEAGE/SPECIES-SPECIFIC EXPANSION OF THE MX GENE FAMILY IN TELEOSTS: DIFFERENTIAL EXPRESSION AND MODULATION OF NINE MX GENES *IN VITRO* AND *IN VIVO* IN RAINBOW TROUT *Oncorhynchus mykiss*

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ABSTRACT
Myxovirus resistance (Mx) proteins are interferon (IFN)-inducible Dynamin-like GTPases, with an important role in antiviral immunity. Three Mx genes (Mx1-3) are known in rainbow trout but nine have been reported recently in Atlantic salmon. In this study, an additional six Mx genes were cloned in rainbow trout, present in four chromosome loci. Further bioinformatic analysis suggests the presence of three teleost Mx groups (TMG) with distinct gene organisations. Whilst some teleost lineages possess all three TMGs, percomorphs have only one (TMG1). Salmonid Mx genes belong to TMG1 and TMG2. Synteny analysis suggests that teleost Mx genes may be evolved from three Mx genes present at two ancestral chromosomal loci, as seen in spotted gar, with the 3R duplicated Mx loci retained/lost in a lineage specific manner. The increased salmonid Mx gene copies result mainly from local gene duplications, that also show species specific differences. Trout Mx molecules have been diversified in loop 1 and 4, and in the nuclear localisation signal in loop 4.
The trout Mx genes are differentially expressed in tissues and cell lines. Whilst TMG1 (Mx1-4) were preferentially expressed in blood, TMG2 (Mx5-9) were highly expressed in the intestine. The expression of several Mx genes were increased in pre-feeding and post-feeding fry, perhaps to help fight potential food borne viruses. The expression of most of the trout Mx genes was induced by poly IC *in vitro* and *in vivo*, and by type I IFN. In addition, as seen in Atlantic salmon, notable induction of some isoforms was seen with IFNγ, and several genes showed some modulation with IL-1β. Overall, induction of trout Mx gene expression was gene-, cytokine- and cell line-dependent. These results suggest that salmonids not only possess a large number of Mx proteins but also possess diversified and complex regulatory pathways, which may help fight viral infection and contribute to their success in an anadromous life style.

KEYWORDS
Rainbow trout Oncorhynchus mykiss, Mx gene family, Anti-viral defense, Phylogenetic tree analysis, Interferons

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O-074

THE TRANSFORMING GROWTH FACTOR (TGF)-B FAMILY IN RAINBOW TROUT (*Oncorhynchus mykiss*): CHARACTERIZATION AND EXPRESSION ANALYSIS

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ABSTRACT
TGF-β is an evolutionally conserved cytokine that belongs to a large family of morphogens and growth factors, with three members (TGF-β1-3) present in mammals. Mammalian TGF-β is widely expressed and modulates a large spectrum of biological processes including normal development, carcinogenesis, and immune responses. It is considered an immunosuppressive cytokine but can also promote the differentiation, function, and homeostasis of certain inflammatory populations of T cells, such as T-helper 17 (Th17) cells. TGF-β members are increased in teleosts, and this is assumed to be due to the teleost-wide third whole genome duplication (3R WGD) event. How exactly how many TGF-β members exist, and how many different paralogues are expressed and modulated is unclear in these fish species, including rainbow trout that experienced a 4R WGD. Analysis of the recently released rainbow trout genome (Acc. no. GCF_002163495.1) identified 10 chromosome loci that each encodes a TGF-β, including TGFβ1a and TGFβ1b cloned previously in our group. The cDNAs of the other TGF-β genes were cloned and reveal that rainbow trout possesses three TGF-β1, two TGF-β2, four TGF-β3 and one TGF-β6. This sequence analysis has been extended to other salmonids. Phylogenetic tree analysis suggests that WGD did play a major role in the expansion of the TGF-β family in teleosts. The expression of the ten TGF-β members in trout was comparative examined by real-time reverse-transcription quantitative PCR in tissues, cell lines and during early developmental stages. The TGF-β family members show differential expression in tissues and cell lines, suggesting functional diversification. The expression of TGF-β members was increased during early developmental stages from pre-feeding to post-feeding. Their expression could be modulated in cell lines by pro-inflammatory cytokines and PAMPs in a cell line- and stimulant-dependent manner. These results suggest that teleost TGF-β members may have undergone lineage-specific expansion and reveal further functional diversification in the teleost immune system.

KEYWORDS: Rainbow trout, TGF-β gene family, phylogenetic tree, gene expression, cytokine.

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O-075

PERFORIN-EXPRESSING IGT+ B CELL WITH CYTOTOXIC ACTIVITY, A NOVEL PLAYER IN THE INNATE IMMUNE RESPONSE OF TELEOST FISH.

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ABSTRACT

We have previously reported that IgT plays a key role in teleost fish mucosal immune responses. This immunoglobulin is produced by a subset of B cells that uniquely express membrane IgT. We have also shown an involvement of these cells in innate immunity as they possess a high phagocytic and microbicidal capacity.

To provide further insight into the roles of B cells in immunity, we performed a comparative transcriptome analysis on FACS-sorted IgT+ and IgM+ B cells. To our surprise, the gene that showed the highest differential expression between these two B cell subsets was perforin, a molecule that is not traditionally associated with B cells. Further analysis confirmed that unlike IgM+ B cells, IgT+ B cells expressed high transcript numbers of several perforin isoforms, including, prf1-like-B, prf1-like-C and prf1-like-D. We further confirmed the unique expression of these perforin genes in IgT+ B cell by single cell transcriptome analysis. Besides the gene expression, perforin protein expression by IgT+ B cells was confirmed by immunostaining, using antibodies we generated against several of the trout perforin isoforms. Since perforin is a cytolytic protein that forms pores on the cell membrane of intracellular and extracellular targets, we hypothesized that IgT+ B cells could possess killing activity similar to that of other perforin-expressing cells, including CD8+ T cells and NK cells. To confirm this hypothesis, we tested the potential cytotoxic capacity of IgT+ B cells towards several mammalian cell lines, such as HL-60. Our results show that the killing activity of IgT+ B cells was significantly greater than that of IgM+ B cells. These data demonstrate a previously unrecognized new function for IgT+ B and vertebrate B cells in innate immunity.

KEYWORDS

IgT+ B cell, Perforin, Innate immunity, cytotoxicity

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O-076

RAINBOW TROUT IGM+ B CELLS PREFERENTIALLY RESPOND TO THYMUS-INDEPENDENT ANTIGENS BUT ARE ACTIVATED BY CD40L

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ABSTRACT

In mammals, B cells can be activated by either thymus-dependent (TD) or thymus independent (TI) antigens. TI responses do not require T cell cooperation and are faster in generating antibodies. On the other hand, conventional B cells (B2 cells) are activated in response to TD antigens within the lymphoid follicles, triggering the formation of germinal centers (GCs). Within these sites, the close interaction with T follicular helper cells stimulate B2 cells to divide and differentiate into antibody-secreting cells (ASCs), reaching a terminal state of plasma cell (PC) or into memory B cells. Among the signals involved in T cell / B cell cooperation, the interaction between CD40 expressed on B cells and its ligand (CD40L) transitionally expressed on activated T cells, is of critical importance. In the absence of lymph nodes or GCs, the mechanisms through which teleost B cells mount extrafollicular IgM responses remains mostly unexplored. In this work, we demonstrate that rainbow trout IgM+ B cells, as B2 mammalian cells, respond to CD40L with effects on survival, proliferation and differentiation. Despite this effect, trout IgM+ B cells, when stimulated with different types of antigens, only reached a general activation state in response to thymus-independent 1 (TI-1) antigens such as TNP-LPS. Interestingly, this response against TNP-LPS was blocked by TLR inhibition, suggesting that TLR cross-linking is an essential signal for B cell activation. Finally, when different types of antigens were combined with CD40L to study their capacity to generate synergies, only TI-1 antigens significantly synergized with CD40L. Our results suggest that in lower vertebrates there is not a clear dichotomy between TD and TI responses whereas accumulation of stimulatory signals is the best approach to generate a full activation state.

KEY WORDS

B cells, CD40L, TD response, extrafollicular response, TLR.

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O-077

EFFECT OF NANOENCAPSULATED CLOVE-OIL ANAESTHESIA IN THE PHYSIOLOGICAL RESPONSE AND IMMUNE STATUS OF NILE TILAPIA

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ABSTRACT

In aquaculture, to minimize the negative side-effects produced by the intrinsic and variated fish management practices, required throughout the farming process, the use of anesthetics is recommended. Paradoxically, adverse side effects resulting from the incorrect management of the same in species-specific domesticated fish have been extensively reported. Taking advantage of the aggregate morphology transition of β-cyclodextrins to form a unique lipophilic 3D complex, just recently we have provided the aquaculture industry with a new nanoencapsulated essential clove-oil (CEO+βCD) anesthetic formula. CEO+βCD preparation that if correctly applied displays a high efficiency on the physiological and immunological behavior in aquacultured fish (Lopez-Canovas et al., 2019). In Nile tilapia farming, the characterization of the physiological effects resulting from using the most extended commercial anesthetics is reasonably available. However, to broaden the anesthetic choices in this species here we aim to characterize extensively the effects of a single 20 mg/L optimal dose in water of our CEO+βCD formula to achieve a 2-2 narcosis stage and compare it with the more traditional formulas, namely AQUI-S (Isoeugenol) and MS-222 (Buffered tricaine methanesulphonate). Five groups of nine naïve Nile tilapia of 450 g average weight each in duplicates would be reared in small 100 L tanks for treatment with anesthetics for 3 min. Once the physical effect of each anesthetic is determined, animals in each group will be transferred to individual rearing tanks. On days 1, 3 and 9 after treatment, three animals per tank will be killed by an overdose of the same anesthetic previously used for treatment. In each sampling, whole blood, skin mucus, gills, head kidney, and skeletal muscle would be aseptically collected to conduct biochemical, mass spectrophotometry, chromatography, histology, scanning-electron-microcopy, and RNA-seq transcriptomics and metagenomic analyses followed by qPCR quantification of target genes. Resulting from this study we anticipate obtaining an extensive physical profile in mucosal tissue subjected to the action of the present anesthetics, the molecular immune pathways related, and the bioaccumulation, if any would be revealed. Moreover, a guided protocol for domesticated Nile tilapia using our CEO+βCD formula would be provided that may contribute to ensure and minimize the animal welfare according to an ethical standard while the particular physical and inflammatory affection of anesthetics improved. All data would be provided and discussed along with the Congress.

KEYWORDS
Anesthetics, CEO+βCD complex, Nile Tilapia, qPCR, RNA-seq, SEM

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O-078

FISH THERMOREGULATION AND THE PROMOTION OF CELLULAR ANTIMICROBIAL DEFENSES

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ABSTRACT
Core body temperature impacts molecular and cellular immune responses. Herein we demonstrate that discrete thermoregulatory programs provide fish with a broader range of immunological benefits than previously anticipated. Complementing prior findings on the modulation of immune gene expression, high resolution quantitative monitoring of cell function showed predictable changes to leukocyte recruitment and pathogen killing capacity. Assessment of kinetics of leukocyte infiltration to a challenge site and changes to the distribution of cellular subsets showed changes in the efficiency of the teleost acute inflammatory response. *Ex vivo* characterization of cell function as well as *in vivo* evaluation of host-pathogen interactions pointed to enhancements in immune defenses and pathogen clearance. These have obvious positive implications for fish health and performance, setting the stage for valuable applications in the aquaculture industry.

KEYWORDS
Teleost fish; cellular immunity; thermoregulation; white blood cell function; aquaculture health and performance

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O-079

MORPHOLOGICAL PROPERTIES OF GILL-EPITHELIAL ANTIGEN SAMPLING (GAS) CELLS IN RAINBOW TROUT

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ABSTRACT

Bath-vaccination is a cost-effective technique to apply vaccines to fish. However, mechanisms of antigen uptake and immune recognition on the mucosal surfaces of fish are largely unknown. We have previously reported that bacterial vaccine antigens are taken up by gill epithelial antigen sampling (GAS) cells. GAS cells are characterized by their binding capacity for the lectin *Ulex europaeus* agglutinin 1 (UEA-1), and their properties of taking up inactivated *Aeromonas salmonicida* subsp. *salmonicida* (*A.s.s.*) bacteria. In addition, GAS cells express genes and molecules that are typical for M cells but also for antigen processing and presentation. Recently, we have developed a monoclonal antibody (mAb) against GAS cells (2B4-1) that specifically binds to UEA- 1+ *A.s.s.*+ cells. In the present study, we aimed to investigate the morphological properties of GAS cells using mAb 2B4-1 by light and transmission electron microscopy (TEM).

The epithelial cells of the gills of rainbow trout were dispersed using 10 mM EDTA and stained with UEA-1 and mAb 2B4-1. UEA-1+ 2B4-1+ cells were sorted by flow cytometry and subjected to May-Grünwald Giemsa staining. The sorted cells were also embedded in epoxy resin and analyzed by TEM. Further, the gills removed from a rainbow trout were embedded in Lowicryl K4M and the ultrathin sections were subjected to immune electron microscopy using mAb 2B4-1.

May-Grünwald Giemsa staining revealed that there are two cell types in UEA-1+ 2B4-1+ cells: one with a fragmented or condensed nucleus and many vacuoles, and a second with a relatively large nucleus. In TEM, the first phenotype of the UEA-1+ 2B4- 1+ cells showed spines on their surface, an electron dense cytoplasm, and numerous lysosome- or phagosome-like vacuoles. The second phenotype showed a rather low- density cytoplasm and some vacuoles in the cytoplasm, suggesting that this cell type is not active in terms of antigen uptake and processing. Immune electron microscopy revealed that mAb 2B4-1+ cells located on the surface of the gill epithelial layer. The mAb signals were found on the spines and cytoplasm of UEA-1+ 2B4-1+ cells.

Taken together, these morphological properties of GAS cells in rainbow trout support our previous observations that teleost GAS cells have functions in antigen processing.

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O-080

GCRV TRIGGERS BUT MAJOR OUTER CAPSID PROTEIN VP4 INHIBITS RIG-I MEDIATED INTERFERON RESPONSE

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ABSTRACT
Grass carp (*Ctenopharyngodon idella*) is a very economically important aquaculture species, however, grass carp reovirus (GCRV) has caused severe epidemic outbreaks of hemorrhagic disease and tremendous mortality in grass carp industry. RIG-I-like receptors (RLRs) are critical cytosolic sensors in antiviral immunity, coupling detection of virus infection to interferon (IFN) production. In the present study, mRNA expressions of RLRs, including retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), were significantly up-regulated after GCRV infection. Extensive type I IFN response was activated by RIG-I- and MDA5-induced IFN regulatory factor (IRF) 3 (IRF3) and IRF7 mRNA expressions and total phosphorylation levels. Meanwhile, LGP2 worked at upstream of RIG-I and MDA5, restraining K63- and K48-linked ubiquitination of RIG-I and MDA5 in various degrees. It inhibited synthesis and phosphorylation of IRF3/7, leading to reduce mRNA levels and promoter activities of IFNs and NF-κB_s_._ _G_C_R_V_ _major outer capsid protein VP4 was found to localize in lysosome, early endosome and endoplasmic reticulum. To investigate the proteins that interact with VP4, intact VP4 protein was employed and immunoprecipitation (IP) was performed using VP4 polyclonal antibody. According to the subsequent LC MS/MS, RIG-I was obtained and verified to interact with VP4 by co-IP and bimolecular fluorescence complementation (BiFC). VP4 overexpression observably declined mRNA expressions and promoter activities of RIG-I and downstream key genes in RLR pathway, including IFNs. As a consequence, antiviral effectors were significantly suppressed in mRNA levels and GCRV replication notably increased, resulting in conspicuously intensified cytopathic effect. Knockdown of VP4 obtains opposite effects. Furthermore, transcriptome sequencing of VP4 overexpression CIK cells was carried out, and the results indicated that VP4 may trigger MyD88-dependent toll-like receptor (TLR) signaling pathway. These results collectively revealed that GCRV infection activates RLR pathway, however, VP4 associated with RIG-I suppresses downstream IFN response to evade antiviral immunity. This study lays the foundation for the further anti-dsRNA virus mechanism research of RIG-I in teleost and the strategy of GCRV for evading the host IFN response.

KEYWORDS
Grass carp reovirus (GCRV); Major outer capsid protein; RIG-I-like receptors (RLRs); Interferon (IFN); Grass carp (*Ctenopharyngodon idella*)

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O-081

IL-4/13A AND ITS RECEPTOR SYSTEM IN ATLANTIC SALMON (*Salmo salar*): UPREGULATION OF KEY GENES INVOLVED IN ADAPTIVE IMMUNITY.

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ABSTRACT

Interleukin (IL)-4 and IL-13 play a central role for T helper 2 immune response in mammals. Cell signaling is mediated by the type I receptor formed by IL-4Ra and gC chains, and the type II receptors formed by IL-4Ra and IL-13Ra1. In fish, IL-4 and IL-13 related genes have been found in several fish species, including rainbow trout and Atlantic salmon. In these salmonid species, three paralogues of the IL-4 and IL-13 cytokines have been reported, *il-4/13a*, *il-4/13b1* and *il-4/13b2*. In regard to the receptors, two paralogues of each IL-4/13 receptor chains have been identified in rainbow trout. In Atlantic salmon, we and others have identified 5 genes named *gc1, il-4ra*, *il-13ra1a, il-13ra1b*, and *il-13ra2*. Since Atlantic salmon is an important aquacultured fish species, and also a good model for the study of evolution of the immune system, the aim of this work was to get new insights into the functional role of IL-4/13A and their receptors in salmon. Thus, salmon *il-4/13A* gene was synthetized and cloned in pET15b and recombinant IL-4/13A was produced in *E. coli*. rIL-4/13A was purified, and the activity verified *in vitro*. *In vivo* analysis of the IL-4/13A biological activity was performed in salmon receiving the recombinant cytokine. Effects were compared with those of a control group receiving saline. Transcription expression of marker genes for Th1 and Th2 responses was analyzed in the spleen and head kidney of treated and control fish. Results showed that IL-4/13A induced the expression of its own gene, GATA-3, IFN-g and MHC class II in the head kidney of fish. No changes were observed for IL-10 in the head kidney. Expression did not change for any of the genes tested in the spleen of the IL-4/13A-treated fish. In regard to the receptors, *gc1, il-4ra, il-13ra1a, il-13ra1b and il-13ra2a* transcripts were detected in most lymphoid and non-lymphoid tissues. Full CDS sequences were cloned from RNA of head kidney leukocytes and then sequenced. Structural analysis of the predicted receptor proteins and 3D models allowed the identification of domains and motifs that are conserved in most IL-4 and 13 receptor chains. Interestingly, IL-4/13A upregulated the transcriptional expression of the receptors in the spleen but not in the head kidney of salmon. Results showed that the IL-4/13 system, which in superior vertebrates induces the Th2 responses, is also conserved in Atlantic salmon and seems to control the expression of key genes involved in adaptive immune responses.

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O-082

IRON OVERLOAD ALTERS THE IMMUNE RESPONSE IN ATLANTIC SALMON AND INCREASES THE SUSCEPTIBILITY TO *Piscirickettsia salmonis* INFECTION

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ABSTRACT
Iron is a vital element for life, but high levels can produce deleterious effects for the organism's development. In mammals it has been demonstrated that iron has an important role in immune system. However, iron overload can increase the production of free radicals, inducing negative effects in immune cells. The excess of iron accumulation has also been associated as a key factor for bacterial pathogenesis. Despite the importance of iron regulation in the immune system, the effects of iron overdoses in fish have poorly been study. The aim of this study was to evaluate the transcriptional changes of Atlantic salmon exposed to iron overload and challenged to the intracellular bacterium Piscirickettsia salmonis. Here, fish were injected with 1 and 5 mg of iron dextran and after eight days injected with P. salmonis. Samples of head kidney, liver and spleen were collected for transcriptome analysis at 0 and 8 days post-injection and 12 days post-bacterial challenge. GO enrichment analysis showed a high number of transcripts differently expressed with association to iron transport, response to oxidative stress and immune response. Notably, fish exposed to iron overload showed downregulation of immune-related genes. Furthermore, histological analysis conducted in infected fish groups showed clinical alterations in salmons previously overloaded with iron. GO enrichment analysis in infected fish showed high abundance of genes associated with immune process regulation, negative regulation of cytokines and regulation of apoptotic process. These biological processes were mainly modulated in fish exposed to iron. This study evidences the effects of iron overload associated to fish immune response, revealing novel insights about the importance of iron regulation and its impact over the immune response in teleost fish.

KEYWORDS
Iron overload, Atlantic salmon, transcriptome analysis, immune modulation, P. salmonis.

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O-083

THE EXPRESSION OF TRPV CHANNELS, PROSTAGLANDIN E2 AND PRO-INFLAMMATORY CYTOKINES DURING BEHAVIOURAL FEVER IN FISH

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ABSTRACT

A fever, or increased body temperature, is a symptom of inflammation, which is a complex defense reaction of the organism to pathogenic infections. After pathogens enter the body, immune cells secrete a number of agents, the functions of which stimulate the body to develop a functional immune and fever response. In mammals it is known that PGE2 is the principal mediator of fever. The extent to which PGE2 and other pro-inflammatory cytokines such as TNF-α, IL-6, or IL-1β could be involved in the induction of behavioral fever in fish remains to be clarified. Several members of the transient receptor potential (TRP) family of ion channels have been implicated as transducers of thermal stimuli, including TRPV1 and TRPV2, which are activated by heat. Here we show that members of the TRP family, TRPV1 and TRPV4, may participate in the coordination of temperature sensing during the behavioral fever. To examine the behavioral fever mechanism in *Salmo salar* an infection with IPNV, infectious pancreatic necrosis virus, was carried out by an immersion challenge with 10 x 105 PFU/mL of IPNV. Behavioral fever impacted upon the expression levels of both TRPV1 and TRPV4 mRNAs after the viral challenge and revealed a juxtaposed regulation of TRPV channels. Our results suggest that an increase in the mRNA abundance of TRPV1 is tightly correlated with a significant elevation in the expression of proinflammatory cytokines (IL-1β, IL-6, TNF-α and PGE2) in the Pre-Optic Area (POA) and cytokine release in plasma. Together, these data indicate that the reduction of TRPV4 expression during behavioral fever may contribute to the onset of behavioral fever influencing movement toward higher water temperatures. Our data also suggest an effect of TRPV channels in the regulation of behavioral fever through activation of EP3 receptors in the central nervous system by PGE2 induced by plasma-borne cytokines. These results highlight for first time in mobile ectotherms the key role of pro-inflammatory cytokines and TRPV channels in behavioral fever that likely involves a complex integration of prostaglandin induction, cytokine recognition and temperature sensing.

KEYWORDS

Ectotherm, Behavioral fever, Cytokine, TRP channels and Virus

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O-084

CHARACTERIZATION OF CD3e+ T LYMPHOCYTES IN THE TELEOST *Dicentrarchus labrax* L.

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ABSTRACT

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 CD3e+ 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 CD3e+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.

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KEYWORDS

CD3e, sea bass, polyclonal antibody, immune responses, T cells

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O-085

IDENTIFICATION AND CHARACTERIZATION OF PLASMA-LIKE CELLS IN GRASS CARP

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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

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O-086

FORMULATION OF NEW ADJUVANTS TO BE USED IN FISH VACCINES

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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.
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KEYWORDS
Turbot, vaccine, *Philasterides dicentrarchi*, ulvan, chitosan, immune response

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O-087

FORMULATION OF *A. salmonicida* ADJUVANTED VACCINE FOR RAINBOW TROUT : IMPACT OF THE ADJUVANT OIL ORIGIN

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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

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O-088

LONG-TERM, PROTEOME-SCALE ANALYSIS OF RAINBOW TROUT IMMUNE PROTEINS: IMPLICATIONS FOR AQUACULTURE VACCINE DEVELOPMENT

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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.

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O-089

A LIVE ATTENUATED STRAIN OF HY9901ΔVSCB PROVIDES PROTECTION AGAINST *Vibrio alginolyticus* IN ORANGE SPOTTED GROUPER (*Epinephelus coioides*) MODEL

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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*

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O-090

YEAST AS A PROTEIN SOURCE WITH HEALTH BENEFICIAL PROPERTIES IN DIETS FOR SMOLTIFYING ATLANTIC SALMON (*Salmo salar* L.)

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ABSTRACT

Atlantic salmon (*Salmo salar* L.) as an anadromous fish undergoes a series of physiological, structural and functional changes in transition from freshwater (FW) to seawater (SW). Fish are more susceptible to stress, physical damage and infectious diseases leading to high mortality and significant economic loss for the aquaculture industry. Yeast produced from lignocellulosic biomass has the potential to serve as a high-quality protein source with health beneficial properties, especially during seawater transfer (SWT) period. In this study, we evaluated the effect of adding 25% *Candida utilis* (LYCC7549) yeast in diets for smoltifying salmon, on growth performance and overall health by using morphometry, immunohistochemistry, cytokine enzyme-linked immunosorbent assays (ELISA) and gene expression analysis during and after SWT. A control diet (Control) and a test diet containing 25% yeast (Yeast) were fed to Atlantic salmon for 4 weeks in FW and 4 weeks after transfer to SW. Our results showed that fish fed the Yeast diet during the whole period or during the FW or SW period achieved higher feed intake and higher growth rate than fish fed the Control diet. Morphometry and immunochemistry analysis of distal intestine (DI) showed that yeast modified immunosuppressive responses related to SW acclimation. A decrease in length of simple folds and in number of CD3 labelled cells in the simple folds of DI in fish fed Control diet was observed, while changes were not present in fish fed the Yeast diet. Yeast significantly decreased the secretion of protein level of cytokines in DI (IFNγ, TNFα, IL-1β, IL-8) and tended to decrease the expression of pro-inflammatory cytokines interleukin 1 beta (*Il1b*) and interleukin 8 (*Il8*) on transcriptomic level. Yeast also modulated the gene expression of aquaporin 8 (*aqp8ab*), superoxide dismutase (*sod1*) and major histocompatibility complex 1 (*mhc1)* in DI, suggesting immunomodulation in Yeast fed fish. These findings suggest that *Candida utilis* yeast is a promising protein source with functional properties in diets for smoltifying Atlantic salmon before and after SWT.

KEYWORDS

Atlantic salmon, yeast, smoltification, immune response, histology

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O-091

BEE POLLEN ADDITION IN ZEBRAFISH DIETS AND ITS EFFECT ON IMMUNE RESPONSE AND MICROBIOTA

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ABSTRACT
Apitherapy is recognized from conventional until contemporary treatment methods. Bee pollen, a natural product with high nutritional properties is recommended as dietary supplement due to many immunostimulating functions as antioxidant, antimicrobial and anti-inflammatory potential. Zebrafish, *Danio rerio*, a widely used species as an experimental model for humans and animals research provides advances in many issues, including immunological investigations. The identification of mechanisms involved in fish immunological activity subjected to bee pollen supplementary treatment can provide important unknown information for the recommendation of this product in diets. Also, zebrafish larval stage is a critical moment due to high mortality rates related to immune challenges. Thus, the objective of our study is to better understand how bee pollen addition in zebrafish diets can influence adults and their offspring immunity. Wild-type zebrafish experiments and procedures were performed as approved by the Consejería de Agua, Agricultura, Ganadería y Pesca de la CARM (authorization number #A13170801). Fish diets containing flakes (3% body weight - BW) and live food, Artemia nauplii, were used as control and compared to fish receiving the same diet supplemented with bee pollen (3% BW) coming from northeastern Brazil. Fish diets were administered during 70 days and the animals were spawned weekly. At 3th and 5th week after diet started, zebrafish offspring with 72 hours post fertilization (hpf) were tested for neutrophil migration after tail wounding. Neutrophils were counted at site of injury at 15, 90 and 360 minutes post wounding (mpw). After diet administration period, intestines from a zebrafish group were collected to metagenomics analysis to compare microbiota between both feeding regime. Total RNA extraction from kidney and abdominal organs of another fish group were quantified for immune-related gene expression after Mucormycosis (*Mucor circinelloides*) intraperitoneal infection. Offspring of zebrafish supplemented with bee pollen presented higher ($P<0.05$) neutrophil migration at 360 mpw compared to offspring of fish fed with standard diets in both weeks' analysis. No changes where observed at initial times after larvae tail wound. Neutrophils are pivotal effector cells of innate immunity and can play an essential role in fish against immunological challenges. Bee pollen dietary supplementation may influence recruitment of these cells in zebrafish offspring. Data regarding metagenomics and gene expression analyzes are still being processed.

KEYWORDS
Fish immunology; Natural products; Nutrition; Neutrophil; Metagenomics
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O-092

IMPACT OF DOWN-STREAM PROCESSING OF BAKERS YEAST (*Saccharomyses cerevisiae*) ON IMMUNE RESPONSES IN ATLANTIC SALMON (*Salmo salar*)

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ABSTRACT

The increased demand for high-quality feed ingredients for the aquaculture industry have turned microbial ingredients as viable sources of protein and lipids. However, limited information exist on the effect of different down-stream processing of microbial ingredients on digestibility and immune responses in fish. In the present study, a feeding trial was performed for 30 days using three different down-stream processing for yeast: spray drying, autolysis or cell crushing and the effect of these treatments on digestibility, immune stimulation and microbiota was evaluated.

Results showed that dry matter digestibility ranged from 42.9 to 63.6% on ingredient level, with the lowest level for the spray dried yeast (250 °C) and the highest level for the 16 h autolyzed yeast. Digestibility of protein ranged from 49.5 to 89.7%. Interestingly, 16 h autolyzed yeast induced the secretion of IL-8, while cell crushed yeast induced the secretion of TNFα in distal intestine, analyzed by ELISA. Further, these diets also increased the number of cells expressing IgM in head kidney, suggesting mobilization of immune cells. In parallel, using high-resolution flow cytometry of bacterial shape (forward scatter) and DNA content (DAPI staining) we were able to quantify the number of bacteria forming the microbiota in the gastrointestinal tract. Individual populations of bacteria were phylogenetically homogeneous and their frequencies changed due to the different diets. The results showed highest number of bacteria in fish fed spray dried yeast, and number of bacteria was correlating with digestibility values having increased number of bacteria in DI of fish having decreased digestibility. This innovative method provides a fast and inexpensive tool to interrogate the microbiota on the single-cell level and offer a unique opportunity to isolate and define bacterial populations for further molecular and functional analysis.

In conclusion, different down-stream processing of yeast do have an impact on protein availability and immune response when used as a protein source in fish feed. Furthermore, its impact on microbiota can be monitored using high resolution flow cytometry.

KEYWORDS

Yeast, down-stream processing, immune effect, flow cytometry, microbiota.

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O-093

A COMBINATED IN VIVO – IN VITRO APPROACH TO EVALUATE THE INFLUENCE OF DIETARY PLANT OILS ON INNATE IMMUNE COMPETENCE AND EICOSANOID METABOLISM PROCESS IN COMMON CARP *Cyprinus carpio*

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ABSTRACT

The study aimed to evaluate the influence of dietary pure linseed oil or sesame oil and their mixture in replacing fish oil on innate immune competence and eicosanoid metabolism in common carp (*Cyprinus carpio*) through a combined in vivo – in vitro approach. A batch of 168 carp juveniles with an initial body weight of 100.4 ± 4.7 g were randomly allocated into 12 tanks of 100 L at a density of 14 juveniles per tank. Four iso-nitrogenous (crude protein = 36.9%) and iso-lipidic (10%) diets were prepared from three lipid sources (cod liver oil, CLO as fish oil source; linseed oil, LO; sesame oil, SO and a blend of linseed oil and sesame oil – SLO, v/v, 1/1 as plant oils). Fish were fed to apparent satiation twice a day for 6 weeks. At the end of the feeding period, on day 42, blood plasma was sampled for lysozyme, complement (ACH50) and peroxidase activity analyses whereas head kidney and liver were dissected for analysing the expression of candidate genes involved in immune competence (*lys, nkef, b/c2*); pro-inflammatory response (*cxc, il8*); fatty acid biosynthesis (*elovl5, fads*) and eicosanoid metabolism process (*pla, pge2, lox5*). On day 45, head kidney leukocytes (HKL) and peripheral blood mononuclear cells (PBMC) were isolated and exposed to *Escherichia coli* lipopolysaccharide (LPS) at the dose of 10µg/mL for 24h. Then the culture medium was collected for peroxidase activity assay while cells were used for gene expression. Results showed that SLO diet enhanced the feed utilization in common carp but no differences of survival and growth were found between diets. No differences were found for genes involved in FA biosynthesis on day 42. Plant oil diets did neither alter lysozyme and peroxidase activities nor gene expression levels (*lys, b/c2, cxc, il8* and *nkef*), except for a lower ACH50 for fish fed SO diet. Moreover, they did not affect the expression levels of some genes (*pla, pge2*, and *lox5*) involved in the eicosanoid metabolism process. However, when head kidney leukocytes were exposed to LPS, *lys* expression was up-regulated in LO-fed fish ($P < .05$) while the expression level of *pge2* in SLO groups was higher than in other groups. Peroxidase activity of HKL exposed to LPS was higher than in control and the highest value was also found in SLO-fed group. In conclusion, our results confirmed that the utilization of plant oil in common carp diets did not induce any negative effect in fish growth, survival and immune competence status even if some humoral compounds seemed less effective in SO than in LO fed fish. Moreover, a dietary combination of SO and LO improved the feed utilization efficiency and seemed more effective in inducing a better immunomodulatory response to LPS through a more active eicosanoid metabolism process.

KEYWORDS
Plant oil, eicosanoid metabolism, immunocompetence, common carp, pro-inflammatory response
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O-094

RESPONSES IN POST-PRANDIAL PATTERNS OF PLASMA MACRO-MINERALS AND IMMUNE FUNCTIONS OF JUVENILE RAINBOW TROUT, *Oncorhynchus mykiss* FED DIFFERENT MONOPHOSPHATE SUPPLEMENTS

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ABSTRACT

Application of feed phosphates (mon/di/tri basic) in high plant protein based diet is a common strategy to accurately cover mineral requirements, especially phosphorous (P), for better growth and health of fish. Considering bioavailability of minerals, monobasic form is more available than di or tri basic ones. A small difference in the P bioavailability may have significant effects on the fish performance and the amount of P discharge to the water. So, the evaluation of different monophosphate supplements is very important to develop eco-friendly aquafeeds. Post-prandial plasma mineral levels and measurement of immune responses can serve as an indicator of mineral bio-availability and health status of fish, respectively. A 60 day's feeding trial was conducted to evaluate the effects of different monophosphates on post-prandial pattern of plasma macro-minerals and immune functions in rainbow trout (*Oncorhynchus mykiss*). Four isoproteic (460 g kg−1 protein) experimental diets were formulated by supplementing monocalcium phosphate, monoammonium phosphate, monopotassium phosphate and monosodium phosphate @ 1.14, 1.0, 1.15 and 1.0 % for diet groups MCP, MAP, MKP and MSP respectively. Triplicate groups of fish (18.5 g) were randomly stocked in 100-L fiberglass aquarium at a rate of 26 fish per aquarium and fed to apparent satiation twice daily. The post prandial plasma P levels were significantly influenced by the dietary treatments and the period of time after a single meal. Significant interaction effects were also observed between the dietary treatment and time period. The baseline concentration of P was significantly higher in MAP, MKP and MSP diet groups compared to the MCP group. The other macro minerals (Ca, Mg, K and Na) were only significantly influenced by the postprandial time interval. Significant positive peak of postprandial P absorption in fish fed diet groups MCP and MAP was observed at 1.5 h while MKP and MSP diet groups showed non-significant increment until 6 h. In all dietary groups significant peak concentration of post prandial plasma macro minerals Ca, Mg, K and Na was observed in 1.5, 4.5, 4.5 and 6 h respectively. In terms of immune functions, respiratory burst and complement activities, total serum protein, peroxidase activity and catalase activity, one phosphate supplement could not prove significant relative advantage over others. However, fish fed dietary group MSP showed significantly higher lysozyme activity and it was not significantly different with MKP and MAP diet groups. MCP diet showed significantly lower lysozyme activity. The results of different immune gene expressions will be discussed in the conference.

KEYWORDS
Monophosphates, Post-prandial absorption, macro minerals, immunity, rainbow trout

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O-095

SEARCHING INTEGRATED STRATEGIES FOR THE EVALUATION OF THE PHYSIOLOGICAL STATUS IN FISH FED FUNCTIONAL DIETS: THE EXAMPLE OF SDPP IN GILTHEAD SEA BREAM (*Sparus aurata*)

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ABSTRACT

The sustained development and intensification of production systems may negatively affect the health of fish, compromising the industry productivity. One of the most used strategies for the sustained improvement in production efficiency is the generation of functional diets. In this study, we evaluated the effect of spray-dried porcine plasma (SDPP) as dietary supplement and its impact upon the physiological and defense status in the skin-associated lymphoid tissue (SALT) of gilthead sea bream. To do it, fish were fed a high-content fish meal diet supplemented with 3% SDPP. After 95 days of feeding, the SDPP-supplemented diet showed a higher somatic growth performance compared to those fish fed basal diet. A higher viscerosomatic index and lower perivisceral fat index were also registered in SDPPfed fish. Fillet proximate composition showed no changes in the fatty acid profile neither the lipid peroxidation nor the activity of oxidative stress enzymes. The skin transcriptomics showed differential expression of 194 genes (DEGs) involved in different processes including cell metabolism, gene expression, protein transport, and protein localization to membrane. The skin mucus proteome identified 35 differential synthesized proteins associated to different processes including epidermis & skin development, epidermal cell differentiation, and metabolism. Importantly, when an integrated multi-omics analysis was carried out, other biological processes were identified, including innate immune response and response to stimuli (organic substances, external stimuli). Taken together, our results suggest that (1) the SDPP is a promising feed additive for sea bream giving a beneficial impact on its growth performance; and (2) the multi-omics analysis is an interesting initial strategy for aquatic organisms that greatly helps to understand the biological processes in an integrated physiological context.

KEYWORDS
Animal nutrition, dietary supplements, mucosal-associated lymphoid tissue (MALT), innate immunity.

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O-096

FEEDING STRESS DUE TO SOY BEAN MEAL AS A MODEL FOR THE DEVELOPMENT OF MOLECULAR IMMUNE MARKERS IN RAINBOW TROUT

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ABSTRACT
The increasing intensification of aquaculture practices has led to public debate about the welfare status of cultured fish.
In order to verify the immune status of trout in context of husbandry stress and to investigate the relationship between chronic stress, immunosuppression, husbandry and feeding we established molecular stress markers using a feeding stress experiment with soybean meal. The welfare status of trout was verified by investigation of mRNA expression of different potential stress regulated genes in whole blood to establish a minimal invasive method. A 56-day feeding experiment was carried out. The triplicate fish groups were fed isoenergetic and isonitrogenic feed mixtures in which the fish meal (50 % of the total diet) was replaced by 0 %, 33 %, 66 % and 100 % soybean meal. EDTA blood was collected from the caudal vein of immobilized trout. In total 88 different genes were tested for their suitability in stress detection using a Fluidigm Biomark HD and a Light Cycler System. Specific primers were designed. Regulated genes belonging to the superior signal transduction pathways such as SERPIN G superfamily, intracellular PI3K/actin, Toll-like receptor, NF-κB, MAP kinase and JAK-STAT signal transduction or intracellular pathogen recognition receptors were tested. The mRNA expression of blood cells was tested for different pro- and anti-inflammatory cytokines, chemokines, substances involved in the acute phase reaction, complement cascade or inflammatory reactions, and heat shock proteins. Finally, different marker genes for specific cell populations were investigated. The housekeeping genes β-Actin, EF1 and RPS5 served as internal standards.
Different genes (e.g. SAA, MPO, NOS2, UCP2) emerged as suitable stress and immune markers and therefore as welfare indicators on a molecular level, while some genes (e.g. IL10, IFN, HSP47) revealed no correlation to feeding stress.
The results represent an important basis for a better assessment of animal welfare in trout farming. They are an important first step towards making well-founded, early assessments of chronic feeding stress of trout in the future, which are minimally invasive. So far, the parameters have often been based on observations such as behavior, color changes and such aspects that are difficult to standardize. These results provide a basis for the development of practical detection systems - comparable to a diabetes test.

KEYWORDS
Stress, inflammation, soy bean meal, feeding stress, molecular marker
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O-097

MODULATION OF THE IMMUNE CONDITION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES THROUGH LONG-TERM METHIONINE SUPPLEMENTATION

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ABSTRACT

Methionine is the first limiting AA in fish diets, particularly in those containing high levels of plant protein sources (e.g. soybean). It is also important to highlight that methionine presents a key role in the immune system of many vertebrate animal models, including fish. In this context, the present study aimed to assess, by means of a dose-response trial, the effects of dietary methionine deficiency or supplementation on the European seabass (*Dicentrarchus labrax*) immune status within the context of an alternative feed formulation (i.e. 0 % fish meal; FM). After acclimatization, European seabass juveniles with a mean body weight of 10.34 ± 0.19 g were randomly distributed in 1000L tanks. In a complete randomized design, five treatments were evaluated in triplicate groups: fish meal free diet with 0.65 % methionine in feed (MET0.65, below requirement), 0.85 % methionine in feed (MET0.85, at req.), 1.25 % methionine in feed (MET1.25, above req.) and 1.5 % methionine in feed (MET1.5, above req.) and a high fish meal diet with 1.18 % methionine in feed (FM, above req.).

After two weeks of feeding fish fed a fish meal free diet with an increased methionine dietary content showed an enhanced expression of genes with direct relationship with methionine aminopropylation pathway and cell proliferation. The immune-enhancer role of methionine was more evident after 12 weeks of feeding with an increased percentage of the peripheral neutrophils and a decrease of apoptotic signals at the transcriptional level. This may indicate an enhancement of fish immune status fed a methionine-supplemented (MET1.25 and MET 1.5) diet compared to the MET0.65 and MET0.85 diets.

Even though MET0.85 and FM dietary treatments presented similar methionine contents, the dietary protein source seemed to also modulate the fish immune status. For instance, European seabass fed the MET0.85 diet presented a reduced expression of several immune-related genes compared to fish fed FM diet. The results suggested the possibility that in a practical plant protein based diet scenario the requirement level of methionine could increase, since fish fed both MET1.25 and MET1.5 dietary

treatments as well as the FM diet presented a similar growth performance at the end, and higher than those fish fed MET0.65 or MET0.65 and CTRL, respectively.

KEYWORDS
Amino acids, immunostimulation, fish, plant protein, fish-meal free

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O-098

ADMINISTRATION OF SINGLE VERSUS COMBINED HERBAL EXTRACTS ENHANCES SOME IMMUNE PARAMETERS AND PROTECTS STRIPED CATFISH (*Pangasianodon hypophthalmus*) AGAINST *Edwardsiella ictaluri*

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ABSTRACT

Psidium guajava and *Phyllanthus amarus* are plants well-known in Vietnamese traditional medicine. However, the capacity of these plants in improving the immune system of striped catfish (*Pangasianodon hypophthalmus*) has received less attention. This study aimed to investigate the effects of single versus combined (1: 1, v/v) ethanolic extracts of *P. guajava* and *P. amarus* on immune response and disease resistance of striped catfish against *Edwardsiella ictaluri*. Fish were fed diets with different concentrations of plant extracts including basal diet 0% [B0]; P. guajava 0.08% [Pg0.08], 0.2% [Pg0.2], 0.5% [Pg0.5]; P. amarus 0.08% [Pa0.08], 0.2% [Pa0.2], 0.5% [Pa0.5] and their mixture (v:v) at similar doses [Co0.08, Co0.2 and Co0.5] for 6 weeks. The growth was examined at week 6 (W6); the cellular immune response (reactive oxygen species-ROS and nitric oxide synthase-NOS) and humoral immune responses (lysozyme and complement activities, total immunoglobulin) were examined at W3, W6 post-feeding and after challenge test; challenge test was performed by injection with *E. ictaluri* at W6, and mortalities were recorded over 15 days post-infection. The extracts supplemented diets did not induce any significant growth difference compared to control. Levels of spleen ROS increased statistically in Pa0.2, Pa0.5 and Co0.5 groups at W6 compared to control. After challenge test, the spleen ROS activity was significantly higher in Pa and Co groups than in the control group ($p<0.05$). However, only Pg0.2 group improved remarkably NOS activity in spleen at W3 and W6. Serum lysozyme activity started to increase significantly after 3 weeks of feeding. Moreover, fish fed Pg diets exhibited a remarkable increase in serum lysozyme levels at W6. Similarly, Pg0.2, Pa0.2 and Co0.2 groups markedly enhanced skin mucosal lysozyme level ($p<0.05$). In addition, Pg0.2 group possessed the highest level of serum complement activity compared to control at W3, while Pa0.5 group showed a maximum ACH50 level at W6. Pa0.2, Pg0.5 and Pa0.5 also strongly enhanced the serum total immunoglobulin (Ig) level at W6 compared to control. After six weeks of feeding, the skin mucosal total Ig level increased remarkably in Pa0.08 and Pa0.2 groups compared to control. The dietary supplementation of single versus combined P. guajava and P. amarus extracts could significantly reduce the mortality and increase the disease resistance of striped catfish following challenge with E.ictaluri compared to control. These results suggest

that *P. guajava* and *P. amarus* extracts have the potential to modulate the immune mechanisms and disease resistance of striped catfish, especially at the medium and high concentrations tested.

KEYWORDS
Striped catfish, Pangasianodon hypophthalmus, immune response, disease resistance, plant extract, Psidium guajava and Phyllanthus amarus
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O-099

LIVING PREY AND PUFA-ENRICHED DIETS TO IMPROVE IMMUNE DEFENSES OF ATLANTIC SALMON (*Salmo salar*) FRY DESTINED TO RESTOCKING

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ABSTRACT
Over the last 40 years, the population of Atlantic salmon (*Salmo salar*) in Europe has decreased by more than 80%, and completely disappeared in Belgium, necessitating restocking programs. To restore salmon populations, artificial reproduction from wild salmon breeders is widely used to produce fry or parr that will be released in river. However, the use of large-scale rearing methods derived from intensive fish farming production could reduce the fitness and health of salmon fry, with possible consequences on its adaptability to natural environment after restocking in rivers. Particularly, the change of dietary lipid composition from around 20% to 10% drastically impacts fry physiology. Indeed, lipids play a key role in fry development and some polyunsaturated fatty acids (PUFA) such as eicosapentanoid acid (EPA) are involved in immune defenses. In addition, the use of dry "germ-limited" food in a controlled environment probably reduces the exposure of fry to environmental bacteria known to shape the immune system and that could later lead to weak immune responses to fight pathogens present in the environment.
In this study, we aimed to optimize the diets of fry to improve their immune defenses and thus their ability to survive in the wild. For this, fry were fed six experimental or commercial diets: experimental diets with 12.7% lipids enriched either with EPA (1) or ALA (2), experimental diets with 20% lipids enriched either with EPA (3) or ALA (4), commercial diet (5), commercial diet (70% of energy supply) supplemented by living chironomids (30% of energy supply) containing complex microbiota (6). After 6 weeks of nutritional conditioning, fry were challenged with the pathogen *Aeromonas salmonicida salmonicida* by bathing. The body part of fry was sampled before and 24h after infection and was used for immune gene expression analyses involved in innate immune responses (*mpo*, *mcsfra*, *lysozyme*). Before bacterial infection, the expression of *mpo* and *igm* genes was lower in fry fed chironomids (F) diets compared to all other diets. This suggested that the supplementation of chironomids in diet could differentially modulate the immune system compared to dry diet. The results of immune gene expression after bacterial challenge, still under analysis, should provide information on how this living preys influence immune defense when fry are exposed to a pathogen. In addition, despite no differences were observed before infection between fry fed EPA- or ALA-enriched diets at different lipid percentage, we could expect some differences in immune responses after bacterial challenge as EPA is a direct precursor of leukotriene and prostaglandins production.

KEYWORDS
Living prey diet; PUFA; bacterial challenge; restocking program; *Salmo salar*

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O-100

META ANALYSES OF TRANSCRIPTOME RESPONSES TO INFECTIONS AND STRESS IN ATLANTIC SALMON

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ABSTRACT
Transcriptomics provides comprehensive information on host responses to pathogens, PAMP, antigens and treatments affecting the immune system. The main goal and challenge in data analyses is finding of consistent trends. Identification of transcription signatures (TS) – gene sets with reproducible expression profiles – is of high value. TS are instrumental for functional annotations of genes, interpretation and classification of transcriptome profiles. We have accumulated a large volume of data for Atlantic salmon produced with DNA microarrays, studies included multiple challenges with viruses, bacteria and parasites and various treatments stimulating immune and stress responses, totally 125 experiments and 4464 microarrays. Meta analyses started with selection of representative controlled experiments with large scale transcriptome responses, finding and ranking of genes with expression changes in at least two related experimental series followed with analyses of expression profiles in the entire database. Three large functional groups with several TS in each are presented, gene numbers and five genes with top ranks are indicated. **Virus responsive genes – VRG** (123 genes, *receptor transporting protein 3, viperin, isg15, ifit5, sacsin*) were identified in multiple trials with four viruses (IPNV, PRV, SAV and salmon poxvirus). These genes are equally activated with viruses and PAMPs (poly-IC, CpG, gardiquimod and bacterial DNA) *in vitro* and *in vivo*. In addition to specialized immune genes, VRG include many members with versatile or unknown functions and genes that most likely have changed their roles in higher vertebrates. While viral infection and exposure to PAMP induce the entire group, VRG fall into subgroups under different conditions suggesting a complex regulatory network. Systemic suppression of VRG was observed in fish infected with *Moritella viscosa* and sea lice. **Markers of inflammation** (105 genes, *C-C motif chemokine 4, serum amyloid, mmp 9, neutrophil cytosolic factor 1 and cathelicidin*) were selected by responses to PAMP and wounds. Multiple functional groups and pathways represent different aspects of immune responses, while several transcription signatures correspond to different scenarios of inflammation. **Stress markers** (31 genes, *mmp 9*, *immediate early response 2, junb, c/ebp-b* and *natterin*) were identified in studies of wound healing and exhausting physical load. These genes respond to various stressors in different tissues of Atlantic salmon. Stress component is manifested in various diseases with different magnitude.

KEYWORDS
Atlantic salmon, transcriptome, antiviral response, inflammation, stress

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O-101

GENOMIC AND BIOLOGICAL CHARACTERIZATION OF INHIBITORS AND ACTIVATORS OF THE NF-kB PATHWAY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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ABSTRACT

Bacterial infection on aquaculture facilities are a major problem for productivity and quality of fish production. One of the most common and important pathogens is Aeromonas salmonicida. This Gram-negative bacterium is the causative agent of the disease furunculosis, which had a devastating impact on salmonid aquaculture up until the late 1980's. An efficient vaccination against this pathogen would represent a decisive improvement of healthcare concepts in aquaculture systems. The NF-kB pathway is considered an important target of vaccinations as it is the responsible for the expression of cytokines and other pro-inflammatory molecules. We profiled the expression of several genes involved in the NF-kB pathway and NF-kB-dependent effector genes in rainbow trout after immunization. using multiplex RT-qPCR. NKIRAS1/NKIRAS2, RelA/NFKB1 and PIAS1 were selected as interesting indicator genes. Using CHSE-214 cell line derived from a Chinook salmon (*Oncorhynchus tshawytscha*) embryo, as in vitro model, we transfected these three factors in order to analyze the subcellular localization
of these proteins. Additional luciferase reporter assays showed that NKIRAS1 inhibits the activation of the NFkappa-β pathway following stimulation with different PAMPs. Surprisingly, NKIRAS2 showed the opposite effect as reported in literature for mammals. On trout cells it rather acts as pro-inflammatory molecule which drives the overexpression of cytokines. Furthermore, a panel of genes, related to the immune response, was selected to determine the modulation of gene expression during the immunization and transfections and analyzed with
Fluidigm technology. Results will contribute to better understanding of the role of inhibitors during inflammatory stimuli.
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KEYWORDS

Inhibitory factors; NF-kB; NKIRAS; Rainbow Trout; Furuncolosis

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O-102

AN IMPROVED GENOME ASSEMBLY FOR *Larimichthys crocea* REVEALS HEPCIDIN GENE EXPANSION WITH DIVERSIFIED REGULATION AND FUNCTION

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ABSTRACT

Larimichthys crocea (large yellow croaker) is a type of perciform fish well known for its peculiar physiological properties and economic value. Here, we constructed an improved version of the L. crocea genome assembly, which contained 26,100 protein-coding genes. Twenty-four pseudo-chromosomes of L. crocea were also reconstructed, comprising 90% of the genome assembly. This improved assembly revealed several expansions in gene families associated with olfactory detection, detoxification, and innate immunity. Specifically, six hepcidin genes (LcHamps) were identified in L. crocea, possibly resulting from lineage-specific gene duplication. All LcHamps possessed similar genomic structures and functional domains, but varied substantially with respect to expression pattern, transcriptional regulation, and biological function. LcHamp1 was associated specifically with iron metabolism, while LcHamp2s were functionally diverse, involving in antibacterial activity, antiviral activity, and regulation of intracellular iron metabolism. This functional diversity among gene copies may have allowed L. crocea to adapt to diverse environmental conditions.

KEYWORDS

Larimichthys crocea, Genome, Hepcidin

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O-103

ROLE OF SEA LICE SECRETOME IN HOST-PARASITE INTERACTION: IMMUNE MODULATION OF SHK-1 CELLS EXPOSED TO *Caligus rogercresseyi* SECRETOME.

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ABSTRACT
Caligus rogercresseyi is an ectoparasite that feeds on mucus, blood, and skin of its host. For a successful infestation, sea lice secrete proteins that allow avoiding host response. Among them, it has been described that trypsin and chymotrypsin have strong proteolytic activity in the peritrophic matrix of the intestinal parasite. In relation to the copepod *Lepeophtheirus salmonis*, it has been suggested that one of the strategies to successfully parasitize its host is given by the secretion of molecules such as proteases, prostaglandin synthetase E2 (PGE2) and cathepsin, causing immunodepression in fish. Moreover, from transcriptomic studies of *C. rogercresseyi* developmental stage has been identified secretome-related proteins as cathepsin, trypsin, and serpin highly regulated during the infective stage, copepodid. The aim of this study was to evaluate the effects of *C. rogercresseyi* secretome over salmon immune and stress response by an in vitro approach using SHK1 cell line. Proteins identification and characterization were performed using the transcriptome database of *C. rogercresseyi.* Characterized sequences were cloned into an expression vector, pET30a and expressed in *Escherichia coli* system. Recombinant proteins were purified by His-tag affinity chromatography. SHK1 cell line was stimulated with 25 ng/mL, 50 ng/mL and 100 ng/mL of recombinant proteins for 24 hours. After cells stimulation, cells were collected for RNA extraction for immune-related genes expression analysis by RT-qPCR. A total of two isoforms of cathepsin, serpin, and trypsin were characterized. With a molecular weight of 36.4, 36.3, 43.5, 49, 26.4 and 27 kDa, respectively. After 24 h of stimulation cell damage was observed in all groups exposed to secretome proteins. Furthermore, differences in immune-related genes expression levels were observed among cells exposed to secretome proteins and control group. This study provides novel information associated with host-parasite interactions associated with *C. rogercresseyi* secretome effects on salmon.

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KEYWORDS
Interaction parasite-host, *Caligus rogercresseyi*; secretome, SHK1 cell line, RT-qPCR

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O-104

TLR-MEDIATED TYPE-I INTERFERON PRODUCTION AND THE REGULATORY MECHANISMS IN CARP THROMBOCYTES

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ABSTRACT
In early stage of viral infection, type-I interferons (IFNs) are produced by signaling from innate immune receptors such as Toll-like receptors (TLRs), which recognize virus-specific molecular patterns including nucleic acids. The type-I IFN transcriptions induced by TLRs are regulated via nuclear factor κB (NF-κB) complex and interferon regulatory factors (IRFs), but these details are still unclear in fish. In the present study, we show that thrombocytes in common carp (*Cyprinus carpio*) have a potent ability to produce large amount of IFNs in response to TLR signaling. Magnetic-sorted HB-8 mAb+ carp thrombocytes and negatively sorted other peripheral blood leukocytes (PBLs) were incubated with resiquimod (also called R848, a potent agonist of TLR7/8), followed by qPCR analysis. The expression levels of the common carp type-I IFNs (ccIFN1 and ccIFN2) in thrombocytes were considerably higher compared with that of in other PBLs. Whereas the ccIFN1 expression was relatively lower than the ccIFN2, the R848 stimulant highly upregulated the ccIFN1 expression than ccIFN2. Although typical inflammatory cytokines including interleukin-6 were also upregulated in thrombocytes, the expression levels were still lower than those in other PBLs. These results indicate that activation of carp thrombocytes by R848 inclines immune system toward antiviral response, rather than inflammation. Expression levels of IRF3 and IRF7 were also upregulated by R848, implying that the IFN transcriptions were activated by these IRFs. The expression of the IFNs and inflammatory cytokines were decreased by several NF-κB signaling inhibitors such as BAY11-7082 or phenethyl caffeate, however, sensitivities to each inhibitor were different between the IFNs and other cytokines. In the presence of those inhibitors, the ccIFN2 expression was correlated with the level of IRF3. In contrast, ccIFN1 expressions seem to be linked to IRF7, suggesting that these two IRFs regulates different IFN genes separately. Our finding suggests that fish thrombocytes are important components for antiviral immunity and can be a new target for the strategy of disease control and vaccine development.

KEYWORDS
Thrombocytes, Interferon, TLRs, innate immunity, interferon regulatory factor

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O-105

ACTIVATION OF DExD/H-BOX RNA HELICASES DURING INFECTION OF ZEBRAFISH AND COMMON CARP WITH SPRING VIRAEMIA OF CARP VIRUS (SVCV) AND CHUM SALMON REOVIRUS (CSV)

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ABSTRACT

The innate immune system detects viral infection predominantly by sensing viral nucleic acids in infected cells. The main sensors of viral RNA in cytoplasm are members of RIG-I-like receptors (RLR) such as RIG-I, MDA5 and LGP2. Recently, several non-RLR DExD/H-box RNA helicases have been also shown to play important role in sensing of viral nucleic acids in the cytoplasm and to activate downstream signaling pathways leading to type I interferons (IFN) production and anti-viral response in mammals. However, the mechanisms of action of these RNA helicases are still not fully understood, and their role in the anti-viral immune response in fish has not been studied. In the present work we aimed to study, for the first time in fish, the anti-viral role of DExD/H-box RNA helicases: DDX1, DDX3, DHX9, DDX21 and DHX36 during viral infection of two cyprinid fish: zebrafish (*Danio rerio*) and common carp (*Cyprinus carpio*). We studied expression of DExD/H-box RNA helicases, type I IFNs and antiviral proteins in zebrafish during infection with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV) both *in vitro* (ZF4 cell line) and *in vivo*. Moreover, expression of studied genes was analyzed in common carp during *in vivo* infection with SVCV. *In vitro* studies of both viral models demonstrated a significant up-regulation of the expression of IFN type I genes in ZF4 cell line. However, SVCV did not induce changes in the gene expression of DExD/H-box RNA helicases, up-regulation of the expression of *ddx3*, *dhx9* and *ddx21* was observed in ZF4 cells upon CSV infection. *In vivo* SVCV infection of zebrafish induced a significant up-regulation of *ddx1* and *dhx36* expression while CSV infection induced a significant up-regulation of *ddx1* and *dhx9* expression. In both infection models an up-regulation of the expression of IFN type I genes and interferon stimulated genes (ISG) *mxa* and *vig-1* was observed. In common carp SVCV infection resulted in up-regulation of the expression of *ddx1*, *dhx9* and *ddx21*, IFN type I and *vig-1*. In both zebrafish and common carp, the up-regulation of the gene expression of DExD/H-box RNA helicases correlated with the increase of the viral load and in most of the cases preceded up-regulation of the IFN type I genes expression. In conclusions our data suggest that non-RLR DExD/H-box RNA helicases might be involved in fish in sensing of viral infection and induction of anti-viaral immune response.

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KEYWORDS
DExD/H-box RNA helicases, interferons, *vig-1*, SVCV, CSV

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O-106

THE FISH COAGULATION SYSTEM COULD HELP TO PREVENT INFECTION BY THE CILIATE PARASITE *Philasterides dicentrarchi*

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ABSTRACT

In addition to its role in hemostasis, the coagulation system is involved in defence against pathogens in invertebrates and vertebrates. In mammals, the coagulation system has been shown to participate in entrapping pathogens and activating the early immune response. Although many studies have described different components of the fish coagulation system, there is a lack of information about the importance of the system in host defence against pathogens. In the present study, we showed that injecting turbot (*Scophthalamus maximus*) with the pathogenic ciliate *Philasterides dicentrarchi* generates the formation of macroscopic intraperitoneal clots in the fish. The clots contained abundant, immobilized ciliates, many of which were lysed. We observed that the plasma clots immobilize and kill the ciliates in vitro. However, fish plasma treated with a tetrapeptide known to inhibit fibrinogen/thrombin clotting in mammals killed *P. dicentrarchi* slightly faster than the untreated plasma, although the overall mortality rate was similar. We also found that kaolin, a particulate activator of the intrinsic pathway in mammals, accelerates plasma clotting in turbot. PMA-stimulated neutrophils, living ciliates and several ciliate components (such as cilia, proteases and DNA) also displayed procoagulant activity in vitro. In addition to generating clots in the peritoneal cavity, i.p. injection of ciliates generated massive migration of neutrophils to the peritoneal cavity, with the formation of large cell aggregates and of numerous fibrin-like fibres in the peritoneal exudate, many of which were associated with peritoneal leukocytes and ciliates. Expression of the CD18/CD11b gene, an integrin associated with cell adhesion and the induction of fibrin formation, was upregulated in the peritoneal leukocytes. In conclusion, the results of the present study suggest that the fish coagulation system plays an important role in immobilizing *P. dicentrarchi* during early moments of infection and appears to be an important component of the protection against this pathogen in fish.

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KEYWORDS
Coagulation system; Complement; Fish; Neutrophils; *Philasterides dicentrarchi*; Plasma

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O-107

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) IS LETHAL TO *Flavobacterium psychrophilum* THROUGH MEMBRANE PERMEABILIZATION AND BY PRIMING IMMUNE FUNCTION IN RAINBOW TROUT MACROPHAGES

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ABSTRACT

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide that is widely distributed in mammals and is capable of performing roles as a neurotransmitter, neuromodulator and vasodilator. This polypeptide belongs to the glucagon/secretin superfamily, of which some members have been shown to act as antimicrobial peptides in both mammalian and aquatic organisms. In teleosts, PACAP has been demonstrated to have direct antimicrobial activity against several aquatic pathogens, yet this phenomenon has never been studied throughout a live bacterial infection. The present study focuses on the influence of PACAP on the rainbow trout monocyte/macrophage-like cell line, RTS11, when exposed to the coldwater bacterial pathogen *Flavobacterium psychrophilum*. PACAP was shown to have direct antimicrobial activity on *F. psychrophilum* when grown in both cytophaga broth and cell culture media (L-15). Further, the ability of teleostean PACAP to permeabilize the membrane of an aquatic pathogen, *F. psychrophilum*, was revealed for the first time. The viability of RTS11 when exposed to PACAP was also observed using a trypan blue exclusion assay to determine optimal experimental doses of the antimicrobial peptide. Interestingly, when RTS11 was pre-treated with PACAP for 24 hours before experiencing exposure to live *F. psychrophilum*, growth of the pathogen was severely inhibited in a dose-dependent manner when compared to control cells receiving no PACAP pre-treatment. Relative expression of pro-inflammatory cytokines and PACAP receptors was also observed in RTS11 following PACAP exposure alone and in conjunction with live *F. psychrophilum* challenge. These qRT-PCR findings revealed that PACAP may have a synergistic effect on RTS11 immune function. The results of this study provide evidence that PACAP has immunostimulatory activity on rainbow trout immune cells as well as direct antimicrobial activity against aquatic bacterial pathogens such as *F. psychrophilum*. As there are numerous pathogens that impact the aquaculture industry, PACAP may stimulate the teleost immune system while also providing an efficacious alternative to antibiotic use.

KEYWORDS

Rainbow trout, antimicrobial peptide, PACAP, RTS11, *Flavobacterium psychrophilum*

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O-108

EFFICIENT AND LONG-LASTING PROTECTION AGAINST THE PACIFIC OYSTER MORTALITY SYNDROME THROUGH ANTIVIRAL IMMUNE PRIMING

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ABSTRACT

The major economic and environmental consequences of recurring mortalities affecting the Pacific oyster *Crassostrea gigas* have initiated many research projects aiming at understanding these phenomena. The solutions anticipated to deal with these mortalities are mainly based on mass selection breeding programs but preventive treatments are still lacking. However, over the last decade, studies have been accumulating revealing the adaptive capabilities of innate immunity, the only component of defense mechanisms in invertebrates. Numerous findings have shown that a wide range of invertebrates can develop innate immune memory (also called immune priming) leading to improved survival during a second encounter with a pathogen. In this context, we undertook to study the possibilities of acting against mortalities by stimulating immune capacities of oysters.

In the present study, we show that the exposure of oyster juveniles to an immunostimulant (a viral mimic called poly (I: C)) can lead to enhanced survival capacities (up to 100%) following OsHV-1 infection or during a mortality episode in the field. That protection is specific to viral protection as poly(I:C) fails to protect oyster against a pathogenic bacteria. We also show that this priming phenomenon is durable as it can last more than 4 months suggesting for the first time the existence of mechanisms of immune memory in this invertebrate species. Finally, analysis of the molecular pathways underlying that protection using dual RNAseq, revealed that priming was based on the triggering of a strong and sustained antiviral response limiting replication of the virus, thus allowing the protection of oysters on the long term. Altogether these results bring new insights into the oyster capacities to build an innate immune memory, its adaptive capacities and provide a platform to further explore novel strategies to help mitigate disease threats upon marine bivalves.

KEYWORDS

Immunity, antiviral, OsHV-1, priming, oyster

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O-109

THE IMMUNE RECOGNITION MECHANISMS IN MOLLUCS

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ABSTRACT

Immune recognition is the first step of immune response, which plays key role in immune protection in all organisms. Since the proposal of pattern recognition theory, numerous pattern recognition receptors (PRRs) have been reported, especially in molluscs where expansive PRRs have been identified by genomic prediction. Taken the model bivalve oyster as example, oysters are constantly threatened by the invasion of pathogens and they have developed a sophisticated repertoire of PRRs to recognize diversiform microorganisms. So far, seven PPRs families, such as peptidoglycan recognition proteins (PGRPs), lectins (including C-type lectins, galectins and sialic acid-binding Ig-like lectins), toll-like receptors (TLRs), C1q domain containing proteins (C1qDCs), Gram-negative binding proteins (GNBPs), scavenger receptors (SRs) and fibrinogen-related proteins (FREPs) have been identified in oysters. What's more, some novel PRRs, such as DM9 domain containing proteins (DM9CPs), Caspases, interleukin 17 (IL-17) and arginine kinase have also been characterized from oysters, which expands our knowledge of PRRs in invertebrates. These various PRRs have been partially validated to be different in recognition specificity, down-stream signal pathway and immune effects. Most of the PRRs serve as multi-functional proteins, not only in immune recognition, but also in the elimination of invading microbes. In addition, these PRRs differentially expressed in mucosal immune tissues and systemic circulatory system where immune recognition taking place. And different effects could be induced by mucosal and systemic immune recognition, such as immune memory or immune tolerance. These results uncovered in molluscs have expanded our knowledge about the classical pattern recognition theory, and also provided theoretical basis for vaccine development in mollusc breeding-species in the future.

KEYWORDS

Mollusc; Immune recognition; Pattern recognition theory; Novel patter receptors; Mucosal recognition

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O-110

HEMATOPOIESIS AND REGULATORY SIGNALING IN MOLLUSCS

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ABSTRACT
Hematopoiesis is a complex process by which different blood cells are formed and released from hematopoietic tissues. Due to lack of oxygen-carrying erythrocytes and blood cells of the lymphoid lineage, which are participating in adaptive immune defense, hematopoiesis in invertebrates offers a simple model system to study regulation of the blood cells of the innate immune system. Several transcription factors have been characterized as hemocyte-specific markers in molluscs, such as Tal-1/SCL, GATA2/3, Runx, CBFβ, ETS, and c-Myb, and are conserved across taxonomic groups from molluscs to chordates. They were highly distributed in the hemocytes as well as potential hematopoietic tissue gill, and the RNAi of Tal-1/SCL, GATA2/3 and Runx significantly reduced the hemocyte renewal rates in the hemocytes and gill tissue. The temporal and spatial expression pattern revealed the potential developmental events of hematopoiesis during ontogenesis of oyster, which initially occurred early in blastula stage and definitively resided in the dorsal region in trochophore larvae. A cytokine-like factor astakine was identified from Pacific oyster *Crassostrea gigas*, which could induce the regeneration of oyster hemocytes either receiving an injection of r*Cg*Astakine *in vivo*, or incubation with r*Cg*Astakine *in vitro*. Furthermore, critical components in signaling pathways, such as Notch signaling pathway, Wnt signaling pathway, were restricted to the potential hematopoiesis sites in the adult oyster, which hints at a possible role for them during the hematopoiesis. In oyster, three types of hemocytes were morphologically identified and separated as agranulocytes, semi-granulocytes and granulocytes by flow cytometry and Percoll® density gradient centrifugation. The granulocytes were proved to be the main immunocompetent hemocytes, and there was potential differentiation relationship among these three sub-population hemocytes. Several hemocyte-specific molecules, such as *Cg*AATase, *Cg*SPSB3, *Cg*CD-9 were identified, which could be employed as a potential marker for the isolation of each subtype hemocytes. Above researches of molluscan hematopoiesis may shed light on the study regulation of the blood cells of the innate immune system in invertebrates.

KEYWORDS
Hematopoiesis; Mollusc; Transcription factors; Cytokines; Hemocyte-specific molecules;

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O-111

DEEP TRANSCRIPTOME PROFILING SHEDS LIGHT ON KEY PLAYERS IN NUCLEUS IMPLANTATION INDUCED IMMUNE RESPONSE IN THE PEARL OYSTER *Pinctada martensii*

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ABSTRACT
Immunological rejection of the pearl oysters following nucleus implantation is a major issue limiting the successful rate of cultured pearls. To date, the molecular mechanism of immune tolerance during pearl formation in the pearl oysters is still largely unknown. Through the RNA sequencing platform and comparative transcriptomic analysis, we investigated the chronic gene expression changes at seven time points (0, 5, 10, 15, 20, 30, 60 days post implantation or dpi) over a period of 60 days following nucleus implantation in the pearl oyster Pinctada martensii. A total of 81,390 unique transcripts (or unigenes) with a combined length of 96.8 million bp and a N50 value of 2,227 bp were obtained. When compared with sequences in the nr, nt, Swiss-Prot, KEGG, COG and GO databases, 36,380 unigenes can find homologous genes. Pairwise comparison of gene expression among all the samples showed that the largest number (or 6,846) of differentially expressed genes was observed at 10 dpi. The number then decreased to below 5,000 at 15, 20 and 30 dpi and increased again to 6,679 at 60 dpi. PCA analysis further showed that the seven time points can be roughly divided into four groups. Comparative transcriptomic analysis between the four groups identified a variety of genes showing differential expression at different time points, including many immune-related genes such as those encoding for toll-like receptor, lectin, scavenger receptor, and peroxidase. In addition, GO and KEGG enrichment analysis revealed that these differentially expressed genes were mainly associated with metabolism, ribosome function, immune response, signaling transduction, and cytoskeleton organization. Notably, two KEGG pathways, namely “cell adhesion molecules” and “primary immunodeficiency” were significantly enriched during the whole process. This finding indicates that genes in these pathways are likely to play critical roles in the immune tolerance of the pearl oysters. To conclude, the data obtained contribute to a better understanding of the molecular mechanisms of allograft induced immune response in the Pearl oysters, and will facilitate the development of effective measures to improve the performance of pearl culture.

KEYWORDS
Pearl oyster, *Pinctada martensii*, Nucleus implantation, Allograft, Transcriptome
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IMMUNE RESPONSES OF AMERICAN OYSTERS TO BACTERIAL AND PARASITIC CHALLENGE

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ABSTRACT

The American oyster Crassostrea virginica is an ecologically and economically important species in the Northwest Atlantic and the Gulf of Mexico. Wild and cultured populations of this organism are impacted by a variety of bacterial and parasitic pathogens. Taking advantage of the recently assembled sequence of the American oyster, we have performed a transcriptomic characterization of the immune responses of oysters to bacterial and parasitic challenge. Evaluation of the response of *C. virginica* larvae to probiotics *Bacillus pumilus* RI0695 and *Phaeobacter inhibens* S4 showed 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

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O-113

CHANGES IN THE HOST MICROBIOTA STRUCTURE AND DIVERSITY DURING THE IMMUNE RESPONSE OF THE SCALLOP *Argopecten purpuratus*

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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
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O-114

PROTEOMIC PROFILE AND PROTEASES CHARACTERISATION OF GREATER AMBERJACK SKIN MUCUS AFTER *Neobenedenia girellae* INFECTION

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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

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O-115

COMPARATIVE TRANSCRIPTOME ANALYSIS OF PILCHARD ORTHOMYXOVIRUS (POMV) AND INFECTIOUS SALMON ANAEMIA VIRUS (ISAV)

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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.

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O-116

CHARACTERIZATION OF FLOUNDER (Paralichthys olivaceus) CD4+ T LYMPHOCYTE SUBSETS IN RESPONSE TO TH-TYPE ANTIGENS

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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;

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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

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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 percent survival in relation to fish fed control diet. PHYTO diet reinforced fish capacity of stress response via protection of head kidney leucocytes from stress-related apoptotic processes. Additionally, dietary supplementation with GMOS and PHYTO compounds increased fish serum lysozime and peroxidase activities.

KEYWORDS

Functional feeds, prebiotics, phytogenics, stress response, immune response

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O-118

DIETARY PREBIOTICS AND PHYTOGENICS IN LOW FISH MEAL AND FISH OIL DIETS FOR *Dicentrarchus labrax*: AN EFFECTIVE TOOL TO GUT HEALTH AND DISEASE RESISTANCE

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ABSTRACT

Fish intestinal mucosal surface supposes a potential route of entrance for pathogenic bacteria. An inflammatory gut reaction can be induced by a variety of factors, such as infection, stress or changes in feed composition. Particularly, for European sea bass (*Dicentrarchus labrax*) feeding low fishmeal (FM) and fish oil (FO) diets results in a gut inflammation-like status. The use of functional additives such as prebiotics and phytogenics simultaneously with a low FM/FO based diet may help to buffer these associated gut health negative-side effects. Four low FM/FO (10%/6%) diets for European sea bass containing galactomannan oligosaccharides (GMOS), a mixture of garlic oil and labiatae oils (PHYTO) were fed for 63 days before exposition to an intestinal *Vibrio anguilarum* infection in combination with a crowding stress. In order to evaluate functional diets efficacy in terms of gut mucosal health maintenance, structural, cellular and immune intestinal status was evaluated by optical and electron microscopy and gene expression analyses. A semi-automated software was adapted to determine variations on goblet cells area and mucosal mucus coverage along the challenge test. Functional diets fed did not affect growth performance, however PHYTO and GMOS dietary inclusion reduced European sea bass susceptibility to *V. anguillarum* after 7 days of challenge test. Rectum (post-ileorectal valve) presented longer ($p=0.001$) folds than posterior gut (pre-ileorectal valve), whereas posterior gut presented thicker submucosa ($p=0.001$) and higher mucus coverage as a result of an increased cell density compared to rectum. Functional diets did not affect mucosal folds length or the grade of granulocytes and lymphocytes infiltration in both intestinal segments. However, fish fed GMOS ($F=14.53$; $p=0.001$) and PHYTO ($F=5.52$; $p=0.019$) presented less posterior gut fold area covered by goblet cells. PHYTO ($F=3.95$; $p=0.049$) reduced posterior gut goblet cell size and increased rodlet cells density ($F=3.604$; $p=0.068$). Dietary GMOS reduced submucosa thickness ($F=51.31$; $p=0.001$) and increased rodlet cells density ($F=3.604$; $p=0.068$) in rectum. Structural TEM analyses revealed a normal intestinal morphological pattern. GMOS increased rectum microvilli length. PHYTO increased ($p\leq0.10$) *Ocln*, *N-Cad* and *Cad-17* posterior gut gene expression. After bacterial intestinal inoculation posterior gut of fish fed PHYTO responded in a more controlled and belated way in terms of goblet cell size and mucus coverage in comparison to other treatments. Rectum pattern of response was similar for all dietary treatments

KEYWORDS
European sea bass, functional additives, gut health, mucus production, disease resistance

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HEALTH STATUS OF SENEGALESE SOLE (*Solea senegalensis*) POST-LARVAE FED DIETS WITH MICROALGAE INCLUSION

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ABSTRACT

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species targeted for aquaculture diversification in Southern-European countries and, as most farmed fish, are potentially subjected to stress and pathogens due to environmental factors (Reis et al., 2017; Pinto et al., 2018). Previous studies reported that algae can be used as antioxidant additive, high-quality dietary protein or source of bioactive compounds, thus promoting optimal growth and health in farmed fish (Becker, 2007; Teimouri et al., 2013). For these reasons, this study intended to evaluate the effects of dietary microalgae inclusion in both health status and growth performance of Senegalese sole post-larvae. Individuals with 41 days after hatching (DAH) were randomly distributed among 12 tanks with an initial density of 3000 post-larvae/m2 and four experimental diets were randomly distributed by triplicate groups of tanks. Three experimental diets (CHLO-*Chlorella* sp., fermented; PHAEO-*Phaeodactylum* sp. and NANNO-*Nannochloropsis* sp.) were formulated to include 3% of each algal biomass to a basal diet, which served as CONTROL. The experimental diets were supplied through automatic feeders set up to supply 8 meals in a 24 h period. At 50 DAH, 20 post-larvae/tank were collected for analysis of immune and oxidative status. Also, at 61 DAH the total length, dry weight and survival were assessed. Homogenates had to be performed for the analyses of immune (i.e. lysozyme and protease activities) and oxidative stress (i.e. catalase activity) related parameters. Survival, relative growth rate and total length of individuals, at 61 DAH were not altered by the dietary treatments. However, post-larvae fed NANNO and CHLO dietary treatments increased dry weight at 61 DAH compared to those fed the CONTROL diet. Neither immune or oxidative stress status were altered by dietary treatments. According to these results, *Nannochloropsis* sp. and *Chlorella* sp. are potential candidates for inclusion in microdiets for Senegalese sole. Further analyses are being carried out to confirm the bioactive potential of these biomasses and optimal dietary inclusion levels.

KEYWORDS
Early feeding, Nannochloropsis, Chlorella, immune status, catalase.

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INTEGRATED TRANSCRIPTOMIC AND FUNCTIONAL IMMUNOLOGICAL APPROACH FOR ASSESSING THE INVASIVENESS OF BIVALVE ALIEN SPECIES.

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ABSTRACT
Biological invasions started when humans moved species beyond their normal geographic limits. Bivalves are the most notoriously invasive species in subtidal aquatic environments. Next-generation sequencing technologies are applied to understand the molecular mechanisms involved in the invasion. The ecological immunology focuses on the role of immunity in invasion, and its magnitude could help to predict the invasiveness of alien species. A remarkable case of invasion has been reported in the Ría de Vigo (Spain) by the black pygmy mussel *Xenostrobus securis*. In Galicia, the Mediterranean mussel *Mytilus galloprovincialis* is the predominant cultured bivalve species. Can we predict the invasiveness of alien bivalve species by analyzing their immune response? Can *X. securis* represent a risk for the autochthonous mussel? We evaluated the suitability of the immune-related hypotheses in our model by using an integrated transcriptomic and functional immunological approach. Our analysis suggests lower immune capabilities in *X. securis* compared to *M. galloprovincialis*, probably due to the relocation of energetic resources from the immune response to vital physiological processes to cope with salinity stress. This multidisciplinary approach will help us understand how the immune response can be influenced by the adaptive process and how this immune response can influence the invasion process.

KEYWORDS
Ecological immunity, invasive species, immune response, mussel, transcriptome.

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TRANSCRIPTOMIC ANALYSIS OF CLAM EXTRA PALLIAL FLUIDS REVEALS IMMUNITY AND CYTOSKELETON ALTERATIONS IN THE FIRST WEEK OF BROWN RING DISEASE DEVELOPMENT

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ABSTRACT

The Brown Ring Disease is an infection caused by the bacterium Vibrio tapetis on the Manila clam *Ruditapes philippinarum*. The process of infection, in the extrapallial fluids of clams, involves alteration of immune functions, in particular on hemocytes which are the cells responsible of phagocytosis. Disorganization of the actin-cytoskeleton in infected clams is a part of what leads to this alteration. This study is the first transcriptomic approach based on collection of extrapallial fluids on living animals experimentally infected by *V. tapetis*. We performed differential expression analysis of transcripts from healthy against infected clams by *V. tapetis*. We highlighted, in infected clams, a downregulation of transcripts implied in immune functions that might suggest an important role of deregulation of lysosomal activity and complement- and lectin-dependent PRR pathways during infection. We have also shown a deregulation of transcripts coding for proteins involved in actin cytoskeleton regulation such as an overexpression of b12-Thymosin (which are actin sequestration proteins) or a downregulation of proteins that closely interact with capping proteins such as Coactosin that countercare action of capping proteins or Profilin. According to our results we made the hypothesis that *V. tapetis* might be able to force hemocytes to stay in a "resting state" to inhibit its phagocytic power.

KEYWORDS

Brown Ring Disease, Hemocytes, Actin cytoskeleton, b-thymosin, Coactosin, Resting cells

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O-122

Vibrio aestuarianus VIRULENT TRAITS : INSIGHTS FROM IN VITRO INTERACTION WITH OYSTER HEMOCYTES

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ABSTRACT
Oyster mortalities associated with pathogenic vibrios are a major concern for the sustainability of oyster farming. Since 2001 and notably since 2011, one bacteria has been regularly detected in France and recently in Ireland: Vibrio aestuarianus. Its implication in oyster mortalities has been validated through experimental pathology and field survey in France4. Moreover, its pathogenesis has been recently elucidated: after a quick colonisation of hemolymph, *V. aestuarianus* virulent strain proliferates and colonizes connective tissues in gills, digestive gland and mantle.
However, shared virulence properties, specific to virulent strains, allowing bacterial proliferation in presence of hemocytes are still poorly described. This study aimed to determine *V. Aestuarianus* virulence strategies, exploring in vitro interactions between bacteria and hemocytes. Adulte oysters hemocytes were exposed to virulent or non-virulent strains. Firstly, to identify commun phenotypic trait we compared hemocyte response face to 8 virulent and non-virulent strains. And secondly, in a more targeted approach, a virulent strain (12/016) and its non-virulent mutant (12/016dVars, previously described) were also compared. Kinetics of hemocytes responses (phagocytosis, mortality and ROS production) were mesured by flow cytometry. Moreover, bacterial proliferation in hemolymph was also estimated for virulent and non-virulent strains and will be presented.

KEYWORDS
Vibrio aestuarianus, oysters, hemocytes, in vitro, phagocytosis.

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IMMUNE RESPONSE OF COMMON CARP TO PRESPOROGONIC DEVELOPMENT OF MYXOZOAN *Sphaerospora molnari*

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ABSTRACT

Sphaerospora molnari is a myxozoan parasite causing skin and gill sphaerosporosis in common carp (*Cyprinus carpio*) in Central Europe. Prior to spore formation, multicellular proliferative stages of *S. molnari* circulate for several weeks in the vascular system of its host despite the array of humoral and cellular immune effector mechanisms. Using our laboratory infection model, we aimed to elucidate the kinetics of presporogonic development of *S. molnari*, while simultaneously analyze the immune responses over a period of 63 days. The obtained results identified two peaks of acute parasitemia on day 28 and 42 respectively. Unexpectedly, the highest parasite load was detected in the liver, a previously unknown localization of *S. molnari*. In response to the infection, the immune system induced dynamic changes in the expression of pro- and anti-inflammatory cytokines, with a predominant role of IL-10 reaching up to 1456 fold increase compared to control fish. The haematological analysis revealed a steady increase in the number of lymphocytes from day 28 onwards, correlating with the growing number of parasites, and only marginal changes in other populations. Additionally, our data revealed a strong increase in the expression of IgM transcripts and increased number of IgM^+ B lymphocytes, which produce specific antibodies recognizing *S. molnari* antigens in western blot. Strikingly, although the sera of infected fish exhibit potent opsonizing capacity *in vitro, S. molnari* isolated from the blood of infected fish are not labelled with carp IgM. These findings indicate the presence of so far unknown evasion strategy and questions the importance of *S. molnari*-specific antibodies in parasite elimination. To our knowledge, this is the first study analyzing the early myxozoan development and immune modulation mechanisms along with innate and adaptive immune responses of the fish host in a controlled laboratory system, adding important information on host-parasite interaction of early metazoans (Cnidaria) with basic vertebrate immune systems.

KEYWORDS
Sphaerospora molnari, Antibodies. Cytokines, B lymphocytes, IL-10

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O-124

FROM GROSS MORPHOLOGY TO GILL TRANSCRIPTOME IN FARMED ATLANTIC SALMON (*Salmo salar*): LESSONS FROM MULTI-SITE SAMPLING

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ABSTRACT

The gill is a multifunctional organ involved in many physiological processes such as gas exchange, osmotic and ionic regulation, acid-base balance and excretion of nitrogenous waste. Due to its interface with the environment, the gill plays a key role as primary mucosal defence tissue against pathogens and is equipped with the gill-associated lymphoid tissue (GIALT). In recent years, the prevalence of gill damage and gill diseases has increased significantly, leading to the substantial losses in Atlantic salmon aquaculture worldwide. Both the transition from healthy to unhealthy gill phenotypes as well the progression of various gill pathologies such as proliferative gill disease (PGD), amoebic gill disease (AGD) and complex gill disease (CGD) are commonly characterised by inflammation and epithelial cell hyperplasia. Routine monitoring for PGD relies on gross (macroscopic) evaluation of gill health, coupled with histological examination of gill sections. To explore underlying molecular events that are associated with progression of PGD, we examined Atlantic salmon from geographically diverse aquaculture sites in Scotland. Total RNA was extracted from 43 fish presenting low or medium gill PGD scores and analysed by whole transcriptome analysis using RNA-seq to determine the molecular signature of the advanced PGD. For each fish, 20M reads were generated and mapped to the Atlantic salmon genome. Importantly, we showed that the sampling site had greater effect on the gill transcriptome than the actual PGD score, providing support for a complex and multifactorial aetiology of PGD, with minimal common molecular responses between different sites. Similar pattern was found for histology, agreeing with the outcome of the RNA-seq analysis. In general, both RNA-seq and histology data clustered together based on the origin of samples, suggesting that the PGD scores may inform about the overall progression of gill damage, but not about the underlying pathology.

KEYWORDS

Proliferative gill disease, gill histopathology, gill transcriptome, PGD scores, RNA-seq

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O-125

A PROTEOMIC STUDY OF RESISTANCE TO BROWN RING DISEASE IN THE MANILA CLAM, *Ruditapes philippinarum*.

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ABSTRACT
Mollusks represent over a fifth of the global aquaculture market, accounting for USD 29.2 billion in 2016. Infectious disease is one of the main limiting factors to the development of mollusk aquaculture, and the difficulties inherent to combating pathogens through antibiotic therapies or disinfection have led to extensive research on host defense mechanisms and host-pathogen relationships. It has become increasingly clear that innate immunity and genetic variability are key factors underlying disease resistance, and that genetic selection for resistance is essential for effective disease control. The Manila clam, *Ruditapes philippinarum*, is a main cultured bivalve species of economic interest produced on a global scale. While the species originates from Japan, it was introduced to European coasts for aquaculture in the early 1970s. In 1987, mass mortalities in clam landings on the Atlantic coast of France led to the identification of Brown Ring Disease (BRD) and of its etiological agent, *Vibrio tapetis*. BRD is characterized by bacterial colonization of the host's extra-pallial compartment, provoking abnormal conchiolin deposits along the inner surface of the shell. While some clams are capable of effectively combating the pathogen, others succumb to a chronic infection characterized by thick conchiolin deposits, low condition index, and death. Although a significant body of research has allowed us to gain a better understanding the pathogen and the disease kinetics, little is known about the molecular mechanisms underlying resistance to this disease.
Within the context of the European H2020 project Vivaldi, an experimental population of Manila clam families was produced, and a batch of juvenile individuals from this population were subjected to a 28-day controlled challenge with *Vibrio tapetis* strain CECT4600. Dual diagnosis was carried out to distinguish between healthy and diseased individuals post-challenge: shells were visually diagnosed for presence of BRD, and a PCR method was adapted to detect *V. tapetis* DNA. Total protein extractions were carried out using whole-body tissue homogenates of healthy and diseased clams and proteins were identified using LC-MS/MS. 2093 protein sequences were matched against a reference transcriptome of the Manila clam, and protein intensities based on label-free quantification were compared to reveal 32 and 55 significant proteins in healthy and diseased clams, respectively. These results provide us with important information regarding the major cell processes and the roles they may play in the resistance of *R. philippinarum* to BRD.

KEYWORDS
Ruditapes philippinarum, Brown Ring disease, proteomics, disease resistance, aquaculture

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O-126

INSIGHTS INTO THE MICROBIOTA OF FARMED AND WILD MYTILUS SP: IS THERE A LINK BETWEEN BACTERIA COMMUNITIES AND HOST SUSCEPTIBILITY?

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ABSTRACT
Since 2014, Mytilus species are affected by mass mortality outbreaks especially in French shellfish farms. A first investigation demonstrated the potential implication of *Vibrio splendidus* through the evidence of a virulent strain10/068 1T1 able to colonize the blue mussel by bypassing external defense barriers and impairing hemocyte activities .
In this study, we explore the role of microbiota in host susceptibility to microbial disease. Different Mytilus species (M. edulis, M. galloprovincialis and hybrid *M. edulis*/*M. galloprovincialis*) were sampled from mussel farms impacted by seasonal mortalities and from natural site. Then, we explored 1) the composition of bacterial microbiota, 2) the mussel susceptibility to the pathogen *V. splendidus* 10/068 1T1 and 3) the impact of Vibrio infection on microbiota bacteria communities.

KEYWORDS
Mytilus sp, microbiota, *V. splendidus*, Vibrio infection

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O-127

GENE ENCODING ENZYMES IN THE UREA CYCLE AND POLYAMINE SYNTHESIS ARE MODULATED DURING AN INFLAMMATORY RESPONSE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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ABSTRACT
The urea cycle and the genes encoding the major enzymes is still poorly characterised in teleost fish and to date there is no information as to how these genes are regulated during inflammation. Central to the urea cycle is the metabolism of arginine and its precursor amino acids, ornithine and citrulline. In salmonids, arginine is an essential amino acid as sufficient quantities cannot be synthesised endogenously and must be obtained in the diet. Arginine has roles in both the inflammatory innate immune response and subsequent tissue healing. To further understand the role of the urea cycle and related cycles (polyamine synthesis and nitric oxide production) in teleosts, we characterised gene families encoding the key enzymes in this pathway, their expression during an inflammatory response and changes in the free amino acid levels in the blood plasma following *Aeromonas salmonicida* challenge. Due to two whole genome duplication events in salmonid evolutionary history, several genes in these pathways have paralogous copies, with divergent expression patterns. The modulation of the genes involved in the urea cycle during inflammation could open up new lines of research for both fish health and nutrition.

KEYWORDS
Urea cycle, Polyamines, Arginine, Inflammation, Functional feeds

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O-128

EFFECT OF DIFFERENT STRESSORS ON THE EXPRESSION OF GLUCOCORTICOID RECEPTOR 1 (GR1) AND GR2 AND THEIR IMPLICATIONS IN THE TRANSCRIPTIONAL IMMUNE RESPONSE IN MUCOSAL SURFACES

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ABSTRACT

Cortisol is considered the most common physiological indicator of stress in fish. It plays a role regulating energy homeostasis, osmotic balance maintenance, and metabolic reorganization. As a consequence, cortisol may compromise the normal function of other biological processes including the host defense through a delay or reduction of the immune response. Its mechanism of action is mediated through glucocorticoid receptors (GRs) in responsive cells. In Perciformes like sea bass (*Dicentrarchus labrax*), two receptors, GR1 and GR2 have been described, but in gilthead sea bream (*Sparus aurata*) there is still little information about them. Thus, the aim of this study was to deep into the characterization of GR sequences of seabream and their expression under chronic and acute stress. For this purpose, sea bream were subjected to a high-intensity chronic stress for 40 days and the sequences for GR1 and GR2 were identified by RT-PCR and sequencing. In agreement with previous data in other Perciformes species, a deletion/insertion in the C domain (DNA-binding region) between GR1 and GR2 was found in the nucleotide sequence. Since there are previous antecedents in fish that GR1 and GR2 respond to different cortisol concentration levels, we also aimed to evaluate whether different stressors (in terms of intensity and duration) may differentially modulate their gene expression. To do it, gilthead sea bream were subjected to an acute stressor (1 min air exposure) monitored at 1, 6 and 24 hours post-stress and to a long-term stressor (two crowding stress conditions: 40 and 70 kg/m3) and their response evaluated at 7 and 14 days post-stress. The implications of the stress response upon the GR1 and GR2 expression and their consequences on the expression of immune-related genes was assessed. A differential expression of GR1 and GR2 under the different stress situations was observed, confirming the presence of two glucocorticoid receptors, and their differential response associated to basal or high cortisol concentrations.

KEYWORDS

Stress, cortisol, Glucocorticoid receptors, mucosal-associated lymphoid tissues, gene expression.

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MORITELLA VISCOSA IN LUMPFISH (*Cyclopterus lumpus*) AND ATLANTIC SALMON (*Salmo salar*)

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ABSTRACT
Winter ulcer disease, caused by *Moritella viscosa*, is a significant problem in cold water salmonid farming, although the bacterium can infect and cause disease in a number of other fish species, such as lumpfish (*Cyclopterus lumpus*). Lumpfish are used as cleaner fish, to eat sea lice from Atlantic salmon (*Salmo salar*) in sea pens. It remains to be established whether *M. viscosa* can be transmitted between the fish species. In this study, we examined whether a salmon isolate of *M. viscosa* could infect and cause disease in lumpfish. We further examined whether a lumpfish isolate of *M. viscosa* could infect and cause disease in salmon. Finally, we examined whether vaccination of salmon with a salmon isolate of *M. viscosa* conferred protection against a lumpfish isolate. The data indicate that while lumpfish appeared to be resistant to a salmon isolate of *M. viscosa*, the salmon could be infected with a lumpfish isolate of *M. viscosa.* Vaccination protected the salmon against the salmon isolate of *M. viscosa* but did not confer sufficient protection to prevent infection with the lumpfish isolate.

KEYWORDS
Atlantic salmon, Lumpfish, *Moritella viscosa*, Vaccine, Experimental infection

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IFIT5 PARTICIPATES IN THE ANTIVIRAL MECHANISMS OF RAINBOW TROUT RED BLOOD CELLS

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ABSTRACT

Recent evidences have demonstrated that rainbow trout nucleated red blood cells (RBCs) halted viral hemorrhagic septicemia rhabdovirus (VHSV) infection inside them. A wide variety of defense mechanisms related to the antiviral immune response have been reported for nucleated RBCs in response to VHSV exposure. In an attempt to identify the specific rainbow trout RBC proteins that interact directly with VHSV, we characterized the immunoprecipitated (IP) proteome of RBCs exposed to VHSV using an antibody against the N protein of VHSV. The IP proteomic characterization identified 31 proteins by mass spectrometry analysis. Among them, we identified interferon-induced protein with tetratricopeptide repeats 5 (IFIT5), a protein belonging to a family of proteins that are induced after the production of type I interferon, which have recently emerged as important players in antiviral innate immunity response. We confirmed the participation of IFIT5 in the rainbow trout RBC antiviral response by examining the expression profile of IFIT5 in RBCs after VHSV exposure at transcriptional and protein levels. In addition, silencing *ifit5* resulted in a significant increase in VHSV replication in RBCs. Moreover, IFIT5 modulation activity assays performed by modulating the IFIT5 RNA-binding pocket cavity, showed an increase in VHSV replication. In summary, these results suggest a possible role of IFIT5 in the antiviral response of RBCs against VHSV. This work broadens the knowledge of fish nucleated RBCs functions and their role in the immune response to viral infections

KEYWORDS

Rainbow trout, IFIT5, red blood cells, VHSV, antiviral, immune response.

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ANALYSIS OF IMMUNE GENE EXPRESSION DURING AGD INFECTION/REINFECTION OF ATLANTIC SALMON (*Salmo salar*)

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ABSTRACT
Farmed Atlantic salmon (*Salmo salar*) is one of the most economically important aquaculture species worldwide. Expansion has increased rapidly since the 1980s, and the subsequent industrial scale of production has led to numerous infectious diseases afflicting the marine grow-out stage. Amoebic gill disease (AGD) is a parasitic disease that was first recorded in Tasmania in 1989 and subsequently in Scotland in 2012, and is characterized by raised white lesions on the gills, with high mortality if left untreated. Current treatment methods primarily consist of freshwater or chemical treatment, and it is often necessary to repeat these treatments for the duration of the marine stage due to regular reinfection. To date, most research on analysis of the immune responses elicited has focused on salmon gene expression after first infection, or alternatively after numerous repeated infection/treatment cycles. This study has compared gene expression in gill and head kidney tissue during early infection (up to 15 days) with early reinfection (up to 14 days) post a single reinfection after hydrogen peroxide treatment. Multivariate analysis of the global transcript expression of a panel of ~40 immune genes showed that in the gill tissue, prior to hydrogen peroxide treatment, pro-inflammatory pathways were stimulated, whereas after treatment, anti-microbial peptides were more highly expressed. In the head kidney, differences between pre and post-treatment sampling points were limited, with the most pronounced changes between 15 days post infection, and 1 day post treatment. This model of reinfection allows differences in the expression of an array of genes between pre and post treatment to be elucidated, and improves our knowledge of how the salmon immune system responds to multiple rounds of AGD infection.

KEYWORDS
AGD, reinfection, gene expression, salmon, Scotland

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WHITE SPOT SYNDROME VIRUS INFECTION IN A CRUSTACEAN

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ABSTRACT
As a lethal pathogen for crustacean aquaculture, the mechanism of white spot syndrome virus (WSSV) infection remains largely unknown. By using a red claw crayfish *Cherax quadricarinatus* primary haematopoietic tissue (Hpt) stem cell culture suitable for WSSSV propagation, we found that the fusion between WSSV and endosome was pH-dependent which was essential for WSSV infection. The internalized WSSV virion was detained into dysfunctional vacuoles in Hpt cells if pretreated by alkalizers, leading to the pH neutralization of endosome system or autophgosome. Importantly, a valosin-containing protein was found to positively regulate the viral delivery with endosome, whose dysfunction resulted in strong aggregation of the intracellular WSSV. When the valosin-containing protein activity was blocked by inhibitor, the co-localization of Cq-GABARAP puncta, an indicator of autophagy activity, on the aggregated virions was significantly increased, indicating the participation of autophagy in the elimination of WSSV. Furthermore, the autophagic sorting and ultimate degradation of the endocytic WSSV were all clearly enhanced in Hpt cells if pretreated with inhibitor, which demonstrated that the autophagy played a defensive role against WSSV infection. Taken together, our data shed new light on the pathogenesis of WSSV which will benefit the antiviral design and breeding selection against WSSV disease in crustacean aquaculture.
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KEYWORDS
Endosome; γ-aminobutyric acid receptor-associated protein (GABARAP); Autophagy; White spot syndrome virus (WSSV); Crustacean

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PROHIBITIN 2 IS ASSOCIATED WITH WSSV INFECTION BY PROMOTING STAT TRANSLOCATION

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ABSTRACT
Prohibitinsare mainly locateat the inner membrane of mitochondrion and that are associated with aging, apoptosis, cancer formation and cell proliferation. In the previousstudies, PHBs were found associated with virus infection and replication by interact with virus proteins. Jak/STAT signalingpathway play a key role in anti-virus immune response. The function of PHBs in the regulation of Jak/STAT remains largely unknown.In the present project, we identified that PcPHB2 was widely distributed in several tissues, and it was induced expression by white spot syndrome virus (WSSV).PcPHB2 significantly promote the amount of WSSV in crayfish, and the quantity of WSSV in PcPHB1 RNAi crayfish was decreased compared with thosein the controls.We further confirmed the interaction of PcPHB2 with the STAT using pulldown and CoIP. Finally, we observed that the protein level of STAT in the nuclear was induced after PcPHB2 injected in the crayfish compared with control. Together, thelocation of STATfrom cytoplasm to nuclear was inhibitedby PcPHB2knockdown. STAT was essential inthe expression of early protein of WSSV. Therefore PcPHB2 is associated with the infection of WSSV by promoting the translocation of STAT.

KEYWORDS
Prohibitin; White spot syndrome virus, Signal transducers and activators of transcription; RNA interference; *Procambarus clarkii*
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COMPARISON OF GENE EXPRESSION IN POST-SMOLT ATLANTIC SALMON CHALLENGED BY LF-89-LIKE AND EM-90-LIKE *Piscirickettsia salmonis* ISOLATES REVEALS DIFFERENCES IN THE IMMUNE RESPONSE ASSOCIATED WITH PATHOGENICITY

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ABSTRACT
Piscirickettsiosis is the main bacterial disease affecting the Chilean salmon farming industry and is responsible for high economic losses. The aim of this study was to describe and comparatively quantify the immune response of post-smolt Atlantic salmon infected by cohabitation with fish bearing LF-89-like and EM-90-like Piscirickettsia salmonis. The expression of 17 genes related to the immune response was studied in head kidney from cohabitant fish by RT-qPCR. Our results at the transcriptomic level suggest that P. salmonis is able to manipulate the kinetics of cytokine production in a way that might constitute a virulence mechanism that promotes intracellular bacterial replication in cells of Atlantic salmon. This strategy involves the creation of an ideal environment for the microorganism based on induction of the inflammatory and IFN-mediated response, modulation of Th1 polarization, reduced antigen processing and presentation, modulation of the evasion of the immune response mediated by CD8+ T cells and promotion of the CD4+ T-cell response during the late stage of infection as a mechanism to escape host defences. This response was significantly exacerbated in fish infected by PS-EM-90 compared with fish infected by PS-LF-89, a finding that is probably associated with the higher pathogenicity of PS-EM-90.

KEYWORDS
Immune response, *Piscirickettsia salmonis*, RT-qPCR

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O-135

THE MICROBIOME AND TRANSCRIPTOME ANALYSIS OF INTESTINAL MICROBIOTA COMMUNITIES IN LARVAL AND DISEASED GROUPER

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ABSTRACT
Grouper, the important economic aquaculture species in Taiwan; however, it is susceptible to infectious diseases such as nervous necrosis virus and Vibrio alginolyticus, and iridovirus during the fry and adult stages. We aimed this researches in aquatic disease control and bioinformatics to investigate the intestinal microbiota community and immunity in grouper by 16S metagenomics and transcriptomic analyses. The results will facilitate establishing database of health-related gut microbial metagenomics in grouper, which can assist in screening for healthy fish fry. Microbiota analysis of *Epinephelus coioides* in metamorphosis stages revealed that dissimilarity in distribution of the individuals in 12dph, 18dph and 50dph. Analysis of immune gene (TLR-3, IL-1β, IFN-α, IFN-γ, NF-kB and Mx gene) showed the fish were in pathogen-free environment. The Proteobacteria in intestine microbiome is most abundant microbes at each development stages. In the other hand, NNV-, GIV-, and Vibrio- infected fish exhibit different phylum composition of microbes. The relationship of pathogen and microbiome in grouper will be investigated in the future, which might be the solution for the grouper disease control.

KEYWORDS
Grouper, intestinal microbiota, development stages, disease, 16S metagenomics
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IDENTIFICATION OF NOVEL BIOMARKERS ASSCOCIATED WITH INFECTION AND CHRONIC STRESS IN RUSSIAN STURGEON

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ABSTRACT

Acipenser spp (sturgeon) are non-teleost fish species of ecological and economic value due to their exquisite meat and caviar. During 20th century, overfishing and pollution have led to a drastic decline of its natural reserves and nowadays many species are listed as critically endangered. Sturgeon aquaculture is thus an important tool for conservation and restoration of sturgeon populations, representing also an activity with valuable socio-economic impact. *A. gueldenstaedtii* (Russian sturgeon), one of the most cultured sturgeon species worldwide, is successfully farmed in Uruguay. However, for achieving a sustainable development, sturgeon aquaculture has to deal with the adaptation of sturgeon to a warmer climate, since increased mortality rates and economic losses are observed during Uruguayan summer. Our previous work demonstrated that warm temperatures (≥24°C) alter fish innate defences by a chronic stress-dependent mechanism. Unfortunately, molecular tools for monitoring chronic stress and innate defences are not commercially available for sturgeons. Therefore, identification of biomarkers associated with inflammation and/or chronic heat stress in sturgeon is crucial for improving sturgeon aquaculture. With this aim, we follow two different experimental approaches: i) analysis of sturgeon acute phase proteins as putative biomarkers of inflammation and ii) analysis by RNA sequencing of differentially expressed genes in sturgeons subjected to chronic heat stress and challenged with pathogenic bacteria. Firstly, we identified several putative acute phase proteins in Russian sturgeon: hepcidin, haptoglobin, hemopexin, transferrin, serum amyloid A protein (SAA) and serum amyloid P protein (SAP). Hepatic expression analysis of these proteins in bacteria-challenged sturgeons showed that SAA could be a good inflammation biomarker candidate. We also obtained specific antibodies against SAA and SAP and analysed their serum protein levels by ELISA or Western blot. We found that SAA, but no SAP levels were significantly increased in serum of bacteria-challenged sturgeons, suggesting that SAA is a positive acute phase protein in Russian sturgeon. However, neither SAA nor SAP showed to be good biomarkers of chronic heat stress. Analysis of differentially expressed genes in liver and spleen of bacteria-infected sturgeons subjected to chronic heat stress are currently being undertaken by Illumina sequencing in order to identify novel inflammation and chronic stress biomarkers.

This identification will open new possibilities for monitoring sturgeon health in farms, contributing to less economic impact from disease outbreaks and favouring aquaculture development.

KEYWORDS: *Acipenser gueldenstaedtii*, infection and chronic-stress biomarkers.

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A LECTIN WITH LDL RECEPTOR DOMAIN SUPPRESS THE REPLICATION OF WSSV

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ABSTRACT
In invertebrates, C-type lectins (CTLs) play important roles in innate immunity against pathogens. Previous studies have focused mainly on the functions of CRD of CTLs in immune recognition, activation, and regulation. Conversely, limited research has been conducted on the relationship of CTLs and low-density lipoprotein (LDL)**.** In this work, a lectin with LDL receptor domain was identified in *Procambarus clarkii* and designated as CTL-R. CTL-R was widely distributed in all tested tissues, and it was induced expression after white spot syndrome virus (WSSV) challenge.
The recombinant CTL-R enhanced the anti-virus action of crayfish, whereas the anti-virus action was inhibited after CTL-R knockdown. The expression level of AMPs were suppress after CTL-R knockdown in crayfish. Furthermore, CTL-R protein induce the activity of dorsal. NF-κB is associated with anti-virus by regulating the expression of AMPs. Therefore CTL-R plays a key role in immune response of WSSV by positive regulation the activity of dorsal.

KEYWORDS
Lectin; White spot syndrome virus; NF-κB; *Procambarus clarkii* ; Innate immunity
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A COMPARATIVE STUDY ON INTESTINAL IMMUNE CELLS IN TWO ELASMOBRANCHS SPECIES

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ABSTRACT
The immune system of fish is of great relevance as it provides information on the evolution of immunity in vertebrates. All sharks, skates and rays are cartilaginous fish and together form a group called the elasmobranchs. Elasmobranchs have a low incidence of disease and their immune cells have been identified as possible sources of novel tumour cell inhibitors. The basic anatomical structure of the elasmobranch gut is similar to that of other vertebrates, with a striking exception of the presence of a spiral intestine that provides an enlarged surface area for digestion and absorption of food by means of spiral folds. During recent years, considerable number of studies have described the immune system of teleosts, whereas little effort has been directed towards studying immunity in elasmobranchs. The lack of knowledge on immune cells in the intestine of elasmobranchs prompted us to carry out present study. During 2018, 13 blackmouth catshark *Galeus melastomus* and 15 specimens of the thornback ray *Raja clavata* were provided by a trawl fleet from the Gulf of Asinara (Sardinia, western Mediterranean Sea). Histochemical, immunohistochemical and ultrastructural observations were conducted on a subsample of spiral intestine of these fish species. Regarding *R. clavata*, three types of granular cells were identified; type I in epithelium, types II and III in lamina propria-submucosa, with each of them containing cytoplasmic granules with distinct ultrastructural characteristics. Immunostaining of intestinal sections showed the reactivity of granular cells: type I cells were positive for lysozyme, mast cell tryptase and tumor necrosis factor-α (TNF-α based on antibody staining; type III cells were immune-reactive to anti-interleukin 6 (IL-6) antibody, whilst type II cells were negative to all the antibodies used. Additionally, in G. melastomus, our studies identified unique, large granular cell type in intestinal epithelium. Electron microscopy showed that the epithelial granular cell type made intimate contact, by means of junctional complexes, with adjacent epithelial and mucous cells. Histochemical staining showed the cytoplasmic granules to be strongly eosinophilic and stained positively to periodic acid-schiff and Alcian blue. Immunostaining of intestinal sections revealed immunoreactivity of the granular cell to TNF-α antibody. However, no reactivity to inducible-nitric oxide synthesis, IL-6, interleukin IL-1β, lysozyme, serotonin 5-HT antibodies was detected. Comparison of each granular cell type in both elasmobranch species with immune cells of teleosts or mammals and a hypothesis on their nature and function would be described.

KEYWORDS
Elasmobranchs; spiral intestine; lysozyme; cytokines; transmission electron microscopy
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Liza ramada INNATE IMMUNITY AGAINST INTESTINAL MICROPARASITE, *Myxobolus mugchelo* (MYXOZOA)

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ABSTRACT

Thinlip mullet Liza ramada is the most abundant of mullet species that live in the Comacchio lagoons (Northern Adriatic Sea, Italy). Histological and ultrastructural sections of the intestine of L. ramada in 30 specimens evaluated, showed that over 80% of specimens were infected with intestinal parasite of mullets, *Myxobolus mugchelo* (Myxozoa). The 'Myxozoa Grassé, 1970', is a phylum composed of over 2400 species. With reference to parasites of mullets, records on myxozoans species encountered in intestine and intestinal mesentery of worldwide mullets revealed that among 12 species of myxosporeans that infect *Liza ramada*, five belong to genus Myxobolus. In histological sections of the infected intestine, plasmodia of *M. Mugchelo* appeared rounded to ovoid and spindle shaped. M. mugchelo conspicuous plasmodia were encysted often in muscle and submucosa layers. In the muscle layer, there was no intense host inflammatory response. Plasmodia containing mature spores were situated closer to mucosal folds and were surrounded by numerous immune cells, mainly mast cells (MCs). Mature spores were generally oval in shape, they were noticed in paracellular space among the enterocytes or within them. Near the infected epithelial cells, several MCs, rodlet cells and few neutrophils were noticed. Indeed, degranulation of the MCs was very frequent in this region. In intestinal epithelium, some large cells with intracellular spores of M. mugchelo, resembling macrophages were documented. Some of these macrophages appeared foamy in aspect and possess elongate striated granules and frequently with enclosed necrotic debris. In some parasitized intestines, sections of epithelium were either completely substituted by spores of M. mugchelo or the spores were noticed inside the damaged enterocytes with spores exuded into the intestinal lumen due to destruction of these cells. These findings enabled us to postulate upon the life cycle of M. mugchelo and the migration of the mature spores through the intestinal wall to reach the lumen. Immunohistochemical analysis on sections of intestinal tissue of uninfected and infected L. ramada revealed positivity of epithelial macrophages to anti-histamine, -leuenkephalin or -serotonin antibodies. The macrophages often showed at the plasma membrane and were seen close to the mucous cells and epithelial cells with intracellular spores of M. mugchelo. In the areas of epithelium infected with spores, epithelial cells positive to proliferating cell nuclear antigen (PCNA) were also observed. The current study is the first record on occurrence of intraepithelial macrophages that engulfed myxozoan spores.

KEYWORDS
Mullet, *Liza ramada*; intestine, microparasite, mast cells, macrophages
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