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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