

weight; serum and whole blood for lysozyme activity and macrophage respiratory burst; gills, gut and hematopoietic organs for histological analysis as well as for expression of interleukin-10. After a 4-weeks experiment, all 4 categories of fish were subjected to experimental challenge with sublethal doses of two pathogens: local Adriatic strain of *T. maritimum* and Mediterranean strain of virus of encephalopathy and retinopathy (VER). One week after the challenge, all fish were sacrificed and same samples as before were taken for analysis, in addition to serum immunoglobulin and tissues for pathogen isolation and identification by PCR (brain for VER and gills for *T. baculum*). Our preliminary results show that although Vetregard did not significantly affect fish growth, different feeding regimes induced different levels of innate immunity reaction.

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### P-362.

#### Characterization of three major cytokines: IL1, TNF1 and TNF2 in reared Atlantic bluefin tuna *Thunnus thynnus*

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#### Abstract

Atlantic bluefin tuna (*Thunnus thynnus*) (BFT) is of great economic significance for Croatian aquaculture and therefore it is necessary to ensure optimal and sustainable conditions during its farming process. Intensive culture of BFT is limited by infectious diseases that, beside the stress, cause heavy losses. However, to date there are no reports of cloning and expression analysis of any major immunity gene of BFT, therefore the objectives of our study were to: report and analyze the full-length sequences of three cytokines with a major roles in innate immunity: tumor necrosis factor alpha 1 and 2 (TNF $\alpha$ 1, TNF $\alpha$ 2) and interleukin 1 beta (IL1 $\beta$ ); develop 3D homology models for BFT two TNF $\alpha$  and IL1 $\beta$  proteins; measure in vivo tissue specific expression of three cytokines in reared BFT during two years of farming process in order to evaluate gene importance as a health biomarker in tuna aquaculture.

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### P-224.

#### Association analysis of the transcriptome and proteome of the shrimp *Fenneropenaeus chinensis* during WSSV acute infection

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#### Abstract

Previous studies have discovered a lot of immune-related genes responding to white spot syndrome virus (WSSV) infection in crustacean. However, little information is available in relation to underlying the mechanism of host responses during WSSV acute infection stage in naturally infected shrimp. In this study, we employed next-generation sequencing and iTRAQ protein quantitative analysis to analyze the transcriptomic and proteomic differences of the shrimp between latent infection stage and acute infection stage. In the transcriptome, a total of 64,188,426 Illumina reads, including 31,685,758 reads from the latent infection group and 32,502,668 reads from the acute infection group, were generated and assembled into 46,676 unigenes (mean length: 676

bp; range: 200–15,094 bp). About 24,000 peptides were predicted and classified based on homology searches, gene ontology, clusters of orthologous groups of proteins and biological pathway mapping. Among which, 805 differentially expressed genes were identified and categorized into 11 groups based on their possible function. Genes in the Toll and IMD pathways, the Ras-activated endocytosis process, the RNA interference pathway, anti-lipopolysaccharide factors and many other genes, were found to be activated in shrimp from latent infection stage turning to acute infection stage. The proPO-activating cascade was firstly uncovered to be probably participated in antiviral process. In the proteome, 3648 unique peptides, derived from 4051 peptides, were identified and 1286 proteins were assembled based on these peptides. Among these proteins, 240 proteins, including 118 up-regulated and 122 down-regulated proteins, were found to be significantly differentially expressed. Component ontology analysis on these proteins showed that up-regulated proteins were mainly enriched in the GO-terms of myosin complex, cytoskeleton, protein complex and so on, while down-regulated proteins were seldom enriched. Association analysis revealed that 72 significantly differentially expressed genes/proteins displayed a same expression trend between the transcriptome and the proteome, including 22 up-regulated genes/proteins and 50 down-regulated genes/proteins, while 14 genes/proteins showed a different expression trend. The data presented in the study will not only provide important information to understand the immune mechanism for shrimp to virus infection, but also provide excellent resource for identifying novel genes in absence of the genome database of shrimp.

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### P-249.

#### IRF10, a novel interferon regulatory factor in zebrafish

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#### Abstract

Interferon regulatory factor (IRF) 10 belongs to IRF family and exists exclusively in bird and fish. Most IRFs have been identified as critical regulators in interferon (IFN) response both in fish and mammals; however, the role of IRF10 in the regulation of IFN expression is still unknown. In this study, we identified IRF10 as a novel factor by which IFN activation was negatively regulated in zebrafish, *Danio rerio*. Firstly, Real-time PCR analysis indicated that zebrafish IRF10 (DrIRF10) was induced by intracellular poly I:C in ZF4 (zebrafish embryo fibroblast-like) cells, suggesting that DrIRF10 is likely a typical IFN-stimulated gene (ISG). Further reporter assay demonstrated that the activations of zebrafish IFN1 (DrIFN1) and IFN3 (DrIFN3) promoters can be inhibited by DrIRF10 in the presence or absence of poly I:C stimulation in EPC (*Epithelioma papulosum cyprinid*) cells, and such inhibition was also observed on the activation of interferon-stimulated response element (ISRE). Meanwhile, overexpression of DrIRF10 was also able to abolish the induction of DrIFN1 and DrIFN3 by the pivotal molecules of Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) signaling pathway. Furthermore, functional domain analysis of DrIRF10 showed that the DNA binding domain (DBD), rather than IRF association domain (IAD), is essential for its inhibitory activity against IFN response. Finally, overexpression of DrIRF10 decreased the transcription level of several IFN-stimulated genes, resulting in the vulnerability of host cells to Spring viraemia of carp virus (SVCV) infection. Collectively, these data suggest that DrIRF10 inhibits the expression of DrIFN1 and DrIFN3 via its DBD domain to avoid excessive immune reaction, which reveals a distinguished regulation mechanism of IFN response in lower vertebrates.

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