crayfish *Cherax quadricarinatus*. *CqTRIM32* was widely distributed in the tissues selected, with the highest expression in muscle, relatively abundant in haematopoietic tissue (Hpt) and the lowest presence in eyestalk. Multiple amino acid alignment showed that *CqTRIM32* contained a conserved RING-finger domain but without B-BOX domain and coiled-coil region, which was different from the traditional TRIMs family. Interestingly, the expression of *CqTRIM32* was significantly up-regulated at both 24 h and 48 h after white spot syndrome virus (WSSV) challenge in *vivo* in crayfish Hpt tissue. Meanwhile, the expression of *CqTRIM32* was significantly up-regulated at both 12 h and 24 h after WSSV challenge in *vitro* in Hpt cells. The quantity of WSSV was increased in red claw crayfish Hpt cell cultures after gene knockdown of *CqTRIM32* post WSSV infection, in which the transcription of both an immediate early gene *ie1* and a late envelope protein gene *vp28* of WSSV were clearly up-regulated. Taken together, our data provide the first evidence that *CqTRIM32* exerts the antiviral activity in a crustacean.

keywords: Tripartite motif-containing (TRIM); Antiviral; White spot syndrome virus (WSSV); Haematopoietic tissue (Hpt); *Cherax quadricarinatus*.

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P-035.

Lipid deposits and foamy macrophage-like cells in focal red and melanised muscle changes in Atlantic salmon (*Salmo salar*)

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Abstract

Focal melanised changes or “black spots” in farmed Atlantic salmon (*Salmo salar*) fillet is a common quality problem seen at slaughter. The changes develop during the seawater phase, starting as acute focal hemorrhages or “red changes” which progress into chronic inflammatory changes with melanisation. Regeneration in most changes remains ongoing without proper healing; a process that has been associated with the chronic persistence and replication of *Piscine orthoreovirus* (PRV).

Another chronically persistent feature in this condition is the histopathological presence of what appears as fat (seen as empty vacuoles) in both focal red and melanised changes. Previous studies have described vacuoles of various sizes assumed to be fat-containing, but as most studies have been carried out on formalin fixed and paraffin-embedded tissues, the content in such vacuoles has diminished during processing and histological investigations of lipids have hitherto been inconclusive.

Here, we use glutaraldehyde-fixed and frozen material, thus preserving the fat. Sections from both acute red and chronic melanised changes were stained with two different special stains (Sudan Black and Oil Red O) for detection of lipids. We show that most vacuoles indeed contain fat and that these are highly prevalent in the acute manifestations in areas of necrosis, haemorrhage and inflammation. We also show fat-containing vacuoles in chronic changes with melanisation, though with a different appearance; often in association with melano-macrophages. In addition, cells though to be foamy macrophages are identified and investigated by transmission electron microscopy. Based on our results, we discuss the potential role of fat in the development of focal melanised changes.

keywords: Inflammation; lipids; Macrophage; Melano-macrophage; Myositis

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P-036.

Immune response in turbot exposed to the ciliate parasite *Philasterides dicentrarchi*

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Abstract

Philasterides dicentrarchi is a marine scuticociliate that causes scuticociliatosis in farmed fish worldwide and is currently considered one of the most important pathogens of cultured flatfish. Although there is abundant information about the infections caused by *P. dicentrarchi* in fish and about how the ciliates and fish immune cells interact in vitro, little is known about the interaction between this ciliate and the fish immune system in vivo. In the present study, turbot (*Scophthalmus maximus*) were exposed twice to the parasite (on days 1 and 21). Immersion infection was performed by adding ciliates to tanks of seawater (18 ºC) to yield a final concentration of 4.5 x 104 ciliates/mL. Fish were exposed to the ciliates by immersion in the seawater for 20 min and were then transferred to tanks of clean seawater for 60 days. Control fish were immersed in seawater with no ciliates, and were subjected to the same conditions as the experimental fish. Four fish died of scuticociliatosis during the experiment. Fish (eight per group) were sampled on days 3, 7, 21 after the first exposure to *P. dicentrarchi* and on days 3, 7 and 40 after the second exposure. The presence of ciliates on the skin and gills was evaluated by qPCR. The IgM, IgT and IgD levels were measured in serum on days 3, 7 and 40 and in mucus on day 40 after the second exposure. Changes in gene expression of immunoglobulins, MHCII and other immune-related genes were determined by qPCR, in gills, skin, and spleen at all sampling times. There were no significant differences in serum IgM, IgD and IgT levels between experimental and control groups at any of the sampling times; however, there was a significant increase in mucus IgT levels 40 days after administration of the second exposure. The results of the qPCR analysis showed few changes of the immunoglobulin expression in the analyzed organs and a mild inflammatory response with the current infective dose.

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keywords: Turbot, *Philasterides dicentrarchi*, immunoglobulins, immune response, infection

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P-037.

Development of a reverse genetics system for snakehead vesiculovirus

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Abstract
Snakehead vesiculovirus (SHVV) is a new rhabdovirus isolated from diseased hybrid snakehead fish (*Channa maculate* ♀ x *Channa argus* ♂) and has caused serious economic losses in snakehead fish culture in China. To better understand the pathogenicity of SHVV, we developed a reverse genetics system for SHVV by using human and fish cells. In detail, human 293T cells were cotransfected with four plasmids encoding the full-length SHVV antigenomic RNA or the supporting proteins including nucleoprotein (N), phosphoprotein (P), and large polymerase (L), followed by the cultivation in Channel catfish ovary (CCO) cells. We also rescued a recombinant SHVV expressing enhanced green fluorescent protein (EGFP), which was inserted into the 3' non-coding region (NCR) of the glycoprotein (G) gene of SHVV. Our study provides a potential tool for unveiling the pathogenicity of SHVV and a template for the rescue of other fish viruses by using both human 293T and fish cells.
keywords: Snakehead vesiculovirus, reverse genetics, EGFP, vaccine

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P-038.

Evidence of trained immunity in teleost fish: Conserved features in carp macrophages

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Abstract
Trained immunity is a form of innate immune memory best described in mice and humans. Trained immunity is defined as a heightened response to a secondary infection that can be exerted toward both homologous and heterologous microorganisms. Typical criteria of trained immunity include: 1) induction upon primary infections or immunizations and subsequent protection against a secondary infection, in a T- and B-lymphocyte independent manner, 2) a response that is less specific than an adaptive immune response but that still confers increased resistance upon reinfection of the host and, 3) the involvement of innate cell types such as NK cells and macrophages involved in improved pathogen recognition and an increased inflammatory response. Clear evidence of the evolutionary conservation of trained immunity in teleost fish is lacking. Given the evolutionary position of teleosts as early vertebrates with a fully developed immune system, we hypothesize that teleost myeloid cells show features of trained immunity common to those observed in mammalian macrophages. These would at least include the ability of fish macrophages to mount heightened responses to a secondary stimulus in a non-specific manner. We established an *in vitro* model to study trained immunity in fish by adapting a well-described culture system of head kidney-derived macrophages of common carp. A soluble NOD-specific ligand and a soluble β-glucan were used to train carp macrophages, after which cells were rested for six days prior to exposure to a secondary stimulus. Unstimulated trained macrophages displayed evidence of metabolic reprogramming, as well as heightened phagocytosis and increased expression of the inflammatory cytokines *IL6* and *TNFα*. Stimulated, trained macrophages showed heightened production of reactive oxygen and nitrogen species as compared to the corresponding stimulated but untrained cells. Measurement of the production of reactive oxygen species proved particularly informative to identify ligands able to train carp macrophages. We discuss the value of our findings for future studies on trained immunity in teleost fish.
keywords: Trained immunity, Cyprinidae, Monocytes/Macrophages, innate immunity,

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P-039.

TLR5 activation site in *Edwardsiella tarda* flagellin is important to induce expression of interleukin-1B and NF-κB genes in Japanese flounder, *Paralichthys olivaceus*

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Abstract
Flagellin is the subunit protein that composes bacterial flagella and is recognized by toll-like receptor 5 (TLR5) as a ligand. Flagellin protein (*e.g.*, FliC and FlaA) contains the D1, D2, and D3 domains; the D1 domain is important for recognition by TLR5 for activation of the innate immune system. In teleosts, there are two types of TLR5, the membrane form (TLR5M) and soluble form (TLR5S), the latter of which is not present in mammals. In this study, the potential of flagellin from *Edwardsiella tarda* (EtFliC) to induce inflammation-related genes interleukin (IL)-1β and NF-κB-p65 through TLR5S in Japanese flounder (*Paralichthys olivaceus*) was elucidated. A transient overexpression system was developed in flounder natural embryonic (HINAE) cells using constructs encoding two flagellin genes derived from *E. tarda* (pEtFliC) and *Escherichia coli* (pEcoFliC) and the flounder TLR5S gene (pPoTLR5S). Expression of inflammation-related genes in EtFliC- and PoTLR5S-overexpressing HINAE cells was significantly lower than in EcoFliC- and PoTLR5S-overexpressing cells. To clarify the difference between EtFliC and EcoFliC potency, the amino acid sequence of EtFliC was compared with that of other bacterial flagellin. The 91st arginine residue, known as the mammalian TLR5 activation site, was conserved in the flagellin of *E. coli* and other bacteria but not in EtFliC. To reveal the importance of the 91st arginine residue in FliC, a pEtFliC construct in which the 91st asparagine was mutated to arginine (pEtFliC_N91R) was generated. Expression of the IL-1β and NF-κB-p65 genes in the HINAE cells co-transfected with pEtFliC_N91R and pPoTLR5S was significantly higher than that in cells co-transfected with pEtFliC and pPoTLR5S. The results suggested that the 91st arginine residue of bacterial flagellin is involved in inflammatory response through TLR5S in teleosts. Thus, EtFliC improved by site-directed mutagenesis could be an effective adjuvant against *E. tarda* infection in Japanese flounder.
keywords: *Edwardsiella tarda*; FliC; TLR5S; IL-1β; NF-κB-p65; Japanese flounder (*Paralichthys olivaceus*)

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