# Monday September 3rd – Gray / Palmer / Pope Ballroom
## Immunology 1 & 2
## Moderators – Susan Fogelson ( Fishhead Labs ) Bartolomeo Gorgoglione ( Wright State University )

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| 9:30 AM | Immunology 1 | Adamek - Antiviral Actions Of 25 Hydroxycholesterol In Fish Cells |
| 9:45 AM | | Maekawa - Analysis Of Immune-Related Genes Expression Response To *Vibrio harveyi* Infection In Orange-Spotted Grouper (*Epinephelus coioides*) |
| 10:00 AM | | Kato - Immune Response Against Mycobacterium Gordonae In *Ginbuna carassius auratus langsdorfii* |
| 10:15 AM | | Li - Internalization Of Large Particles By Turbot (*Scophthalmus maximus*) Igm+ B Cells Mainly Depends On Macropinocytosis |
| 10:30 AM | | **Refreshments** |
| 10:45 AM | Immunology 2 | Braden - Should I Stay Or Should I Go? Testing The Divergent Immune Hypothesis In Steelhead Salmon *Oncorhynchus mykiss* |
| 11:00 AM | | **Kaneko - Uptake And Transportation of Bacterial Antigens In The Rainbow Trout Intestinal Epithelium** |
| 11:15 AM | | **Liu - Autophagy And Infectious Disease In Rainbow Trout** |
| 11:30 AM | | **Mori - Changes In Skin Mucus Protein Profile And Immune-Related Gene Expression In Skin Of Japanese Flounder *Paralichthys olivaceus* Fed A Diet Supplemented With High Concentrations Of Ascorbic Acid** |
| 11:45 AM | | Karsi - Live Attenuated *Edwardsiella ictaluri* Vaccines Stimulate Active Phagocytosis And Bacterial Killing Of Catfish B Cells |

**Antiviral Actions of 25 Hydroxycholesterol in Fish Cells**

Mikolaj Adamek[1*], Jonathan Davies[1,2], Alexander Beck[3], Lisa Jordan[3], Anna Becker[3], Marek Matras[4], Michal Reichert[4], Keith Way[5], Jun Zou[6] and Dieter Steinhagen[1]

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Cholesterol is essential for building and maintaining cell membranes, is the main component of lipid rafts and by this modulates membrane fluidity. This makes cholesterol critical for several steps in the viral replication cycle, especially for enveloped viruses. Virus infections of mammalian cells, lead to the induction of the oxysterol 25 hydroxycholesterol (25HC), a soluble antiviral factor, which is produced from cholesterol by activation of cholesterol 25 hydrolase (CH25H). Immune responses based on CH25H were largely not studied in fish. Therefore, putative genes encoding for CH25H were identified and amplified in common carp and rainbow trout and an HPLC-MS method was established for measuring oxysterols in fish cells. Our results give substantial evidence that CH25H activation is an antiviral response against a very broad spectrum of viruses in both common carp and rainbow trout cells *in vitro*. Furthermore, the *ch25h* expression is also modulated *in vivo* in common carp during viral infections. HPLC-MS analyses showed that even fibroblastic cells are capable of producing 25HC and its metabolite 7a,25diHC. The 25HC had an antiviral activity by blocking the entry of *cyprinid herpesvirus 3* (CyHV-3) but not *spring viremia of carp virus* (SVCV) and common carp paramyxovirus (CCPV) in KFC cells and *viral haemorrhagic septicaemia virus* (VHSV) and *infectious pancreatic necrosis virus* (IPNV) in RTG-2 cells. The stimulation of RTG-2 cells with rainbow trout recombinant type I IFN provided further evidence that despite the fact that CH25H based antiviral response coincides with type I IFN responses; it is not type I IFN dependent. Interestingly, the vulnerability of CyHV-3 to 25HC is counteracted by a downregulation of the *ch25h* gene expression in carp fibroblasts during CyHV-3 infection.

**Conference Session Designation:** ( Immunology General )
**Presentation Format:** ( Oral )

**Analysis of Immune-Related Genes Expression Response to *Vibrio harveyi* Infection in Orange-Spotted Grouper (*Epinephelus coioides*)**

Shun Maekawa[1], Omkar Byadgia[1], Yao-Chung Chen[1], Pei-Chyi Wang[1], Takashi Aoki[1,2], Haruko Takeyama[3], Jun-ichi Hikima[4], and Masahiro Sakai[4] and Shih-Chu Chen[1]

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*Vibrio harveyi* is a Gram-negative fish pathogenic bacterium. To investigate the expression of immune related genes responding to *Vibrio harveyi* infection, in this study, we performed transcriptome analysis of kidney and spleen in orange-spotted grouper (*Epinephelus coioides*) after *Vibrio harveyi* infection. A total of 79,128 unigenes were obtained after de novo assembly using the Illumina sequencing platform. The numbers of total differentially expressed genes (DEGs) in head kidney at 1 dpi, head kidney at 2 dpi, spleen at 1 dpi, and spleen at 2 dpi were 7918, 4260, 7887 and 8952, respectively. The differentially expressed genes were mainly annotated into signal transduction and immune system based on KEGG data base. The DEG were enriched in immune related pathway, such as Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, NF-kappa B signaling pathway and Jak-STAT signaling pathway. In the spleen at 1 and 2 dpi, the expression levels of genes related to NF-kB signaling were up-regulated, including cytokines (IL-lb, RANKL), and NF-kB signaling mediators (Myd88, TAK1, IKKa, p50). These results indicated that NF-kB signaling was activated by *V. harveyi* infection. In the kidney at 2 dpi, while these immune-related genes were down-regulated. Therefore, it is suggested that the spleen is the primary organ for immune responses to *V. harveyi* infection rather than head kidney in orange-spotted grouper. These data will offer the functional analysis of immune-related genes in orange-spotted grouper against *Vibrio harveyi* infection.

**Conference Session Designation:** ( Immunology General )
**Presentation Format:** ( Oral )

**Immune Response Against *Mycobacterium gordonae* in Ginbuna *Carassius auratus langsdorfii***

Goshi Kato[1]*, Taichi Kakazu[1], Mitsuo Yamada[1], Lik-Ming Lau[1], Kazue Nakajima[2], Shoh Sato[2], Teruyuki Nakanishi[3], Makoto Endo[1] and Motohiko Sano[1]

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Mycobacteriosis causes severe economic losses of fish production in aquaculture industry worldwide. Recently, we isolated Mycobacterium gordonae from diseased koi carp from some culture ponds in Niigata, Japan. In this study, we investigated the immune responses against M. gordonae in ginbuna crucian carp as a model fish species. An isogenic clone of ginbuna crucian carp (OB1 clone) were intraperitoneally injected with 2.0 × 107 CFU/fish of M. gordonae and cumulative mortality was recorded for 170 days. The trunk kidney was sampled from ginbuna injected with 1.9 × 108 CFU/fish at 1, 3, 7, 14, 21 and 28 days post-infection. Gene expression levels of CD4-1, CD4-2, CD8α, IFN-γ2 and T-bet in the trunk kidney were determined by quantitative RT-PCR. Furthermore, hematoxylin-eosin (HE), Ziehl-Neelsen (ZN), immunohistochemistry using anti-ginbuna IFN-γ and anti-ginbuna CD4-1 polyclonal antibody were performed using paraffin sections of the trunk kidney samples. Cumulative mortality was 50% in M. grodonae challenged fish at 170 days post-infection, while no mortality was observed in PBS-injected fish. CD4-1 and CD8α gene expression level was significantly up-regulated in M. gordonae-infected fish at 21 and 28 days post-infection, whereas the level of CD4-2 was not changed during the experiment. Gene expression levels of T-bet and IFN-γ2 was up-regulated during 7 to 28 days post-infection in the infected fish, suggesting the dominance of Th1 immunity. Granulomatous responses consisted of central macrophage accumulation and surrounding lymphocytes and ZN-positive bacterial cells were seen through the damaged area. Immunohistochemistry revealed that the marginal lymphocytes were positive for anti-ginbuna CD4-1 antibody, and the IFN-γ producing cells were also located surrounding the mycobacterial cells-laden phagocytes. These results clearly indicate that CD4-1 positive cells participate in granuloma formation in teleost fish. In addition, IFN-γ2 may play important roles in the granulomatous inflammation in teleost fish.

**Conference Session Designation**: ( Immunology General )
**Presentation Format**: ( Oral )

**Internalization of Large Particles by Turbot (*Scophthalmus maximus*) Igm$^+$ B Cells Mainly Depends on Macropinocytosis**

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Increasing evidence has demonstrated support for the endocytic capacities of teleost B cells. In the present study, the ability of turbot IgM$^+$ B cells to ingest microspheres of different sizes and the corresponding internalization pathways were investigated. The results showed that IgM$^+$ B cells exhibited relatively high endocytic capacities for 0.5μm and 1μm latex beads, and that different mechanisms were employed for IgM$^+$ and IgM$^-$ cells to uptake 0.5μm and 1m beads. For 0.5μm beads, IgM$^+$ B cells apparently employed macropinocytosis-dependent endocytic pathway, whereas IgM- cells utilized a different process involving both clathrin- and caveolae-mediated pathways. For the uptake of 1 μm beads, IgM$^+$ cells relied mainly on macropinocytosis and partially on caveolae-mediated pathway, while IgM- cells utilized the routes similar to that of internalizing 0.5μm beads. Consistently, the internalized microspheres were co-localized with high-molecular-mass dextran in IgM$^+$ phagocytic cells. In addition to latex beads, IgM$^+$ B cells could also ingest inactivated bacteria predominately through macropinocytosis and caveolae-mediated endocytosis. These results collectively indicated that macropinocytosis is principally responsible for particle uptake by turbot IgM$^+$ B cells.

**Conference Session Designation**: ( Immunology General )
**Presentation Format**: ( Oral )

**Should I Stay or Should I go? Testing the Divergent Immune Hypothesis in Steelhead Salmon *Oncorhynchus mykiss***

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The process of smoltification in anadromous fishes is extremely energetically demanding, and so a common strategy is to temporarily divert energy from less important physiological systems in order to maximize survival during this stressful transition. Earlier work characterizing the transcriptomic response of rainbow trout, *Oncorhynchus mykiss*, suggested that the immune system of resident (fresh water, rainbow trout) was divergent from that of migratory (anadromous, steelhead salmon) *O. mykiss*. Resident fish showed divergent transcriptomic profiles in the gills compared to migratory fish, with several markers of dendritic cell (DCs) differentially expressed between the groups. However, the cell population responsible for this response in the gills was never characterized or identified. Thus, the aim for this present work was, 1.) to test the divergent immune hypothesis by characterizing more DC markers in an unknown population of *O. mykiss*, and, 2.) to localize and characterize the cells expressing these markers in the gills of this population. To achieve this, equal portions of the right gill arch of ten fish of unknown status (i.e., either resident or migratory) from the same population of the above study (Abernathy Creek, Columbia River system, Washington, US) were dissected from euthanized fish and placed in either RNAlater™ for downstream genetic analysis, or 10% buffered formalin for immunohistochemistry. Resulting transcript abundance of several markers of DCs (*cd83, cd80/86, mh□ii, clec4m,* and *cd209*) and smoltification (*s100a4, nka*) was used as input for principal component and clustering analysis. The data was clearly delineated into two distinct clusters composed of resident or transient fish, with expression of *s100a4* and *nka* positively correlated with expression of immature DC markers *cd209* and *clec4* while negatively correlated with expression of mature DC markers *cd83* and *cd80/86*. Furthermore, we identified $CD83^+$, $CD80/86^+$, and $MH□II^+$ cells in the gills of resident and migratory fish concordant with gene expression. Taken together, this present work supports the existence of a divergent immune strategy by *O. mykiss*, whereby the gills of migratory populations are characterized by immature DCs, while those of resident fresh-water populations are characterized by maturing DCs. The significance of these findings in respect to markers of smoltification and differential pathogen exposure will be discussed.

**Conference session designation:** ( Immunology general )
**Presentation format:** ( Oral )

**Uptake and transportation of bacterial antigens in the rainbow trout intestinal epithelium**

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In mammals, microfold (M) cells and goblet cells can take up bacterial antigen and transport it across the intestinal epithelium. In the teleost intestinal epithelium, M-like cells (lectin binding: UEA-$1^+$ $WGA^-$) and goblet cells (UEA-$1^+$ $WGA^+$) are considered to be antigen-sampling cells. However, the mechanisms underlying uptake and translocation of the bacterial antigen are still unclear. In this study, we investigated the antigen uptake and antigen transportation across the intestinal epithelium in rainbow trout after rectal administration of formalin-inactivated bacteria.

Rainbow trout were rectally administrated with five inactivated bacterial pathogens (*Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio anguillarum* J-O-1, *V. anguillarum* J-O-2, *V. anguillarum* J-O-3 or *Streptococcus iniae*) at a dose of 2.2-5.0 × $10^8$ CFU/ml, and the intestine was sampled at 30 min post-administration. Paraffin-embedded section of the intestine was subjected to immunofluorescent staining with rabbit anti-serum raised against each bacterium and subsequently with either anti-macrophage antibody, UEA-1 or WGA. Furthermore, rainbow trout were rectally administrated with five inactivated bacteria stained with syto61 and the intestine was sampled at 30 min post-administration. The epithelial cells were isolated from the intestine, stained with UEA-1 and analyzed by flow cytometry.

Immunofluorescent staining revealed that the bacterial antigens were taken up by goblet cells (UEA-$1^+$ $WGA^+$) in the intestinal epithelium. FS-SS plot of the intestinal epithelial cells in flow cytometry showed the increase of SS value in UEA-$1^+$ cells after the bacterin administration, suggesting that mucus granules increased in the goblet cells. However, antigen sampling by M-like cells (UEA-$1^+$ $WGA^-$) was not observed in this study. In addition, the inactivated bacterial antigens were also found in the lamina propria and were phagocytosed by macrophages. These results suggest that the goblet cells in the rainbow trout intestinal epithelium mainly take up bacterial antigen and transport it to macrophages in the lamina propria.

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**Presentation Format:** **(** Oral )
**Student Presentation:** ( Yes )

**Autophagy and Infectious Disease in Rainbow Trout**

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Eukaryotic cells normally employ autophagy to degrade cellular components including proteins and organelles. This highly evolutionarily-conserved mechanism is present in eukaryotic cells from yeast to mammals and the most important autophagy-related genes have been identified in some teleosts. Some unfavorable conditions such as nutrient restriction can induce autophagy. Increasing evidence indicates that autophagy is involved with both the innate and adaptive immune responses and that it influences the success of infectious agents. Previous research in our laboratory revealed that feed restriction and deoxynivalenol (DON) reduced the mortality of rainbow trout to experimental *Flavobacterium psychrophilum* infection. The aim of our research is to elucidate the possible role that autophagy plays in rainbow trout resistance to *F. psychrophilum* and to viral hemorrhagic septicaemia virus (VHSV). The first objective was to confirm that autophagy can be regulated by nutritional restriction, selected chemicals and VHSV. Autophagy in rainbow trout gill epithelial cells (RTgill-W1) was induced by VHSV infection, and low serum concentration (2% FBS), but was blocked by chloroquine (CQ) and suppressed by DON. However neither the widely used autophagy inhibitor, 3-methyladenine, nor autophagy activator, rapamycin, acted consistently as expected. Similar *in vitro* results were also observed in a rainbow trout liver cell line, RTL-W1. The second objective was to apply ration restriction and chemical mediators, e.g. CQ and DON to examine their effect on survival in *in vivo* experimental infection trials with *F. psychrophilum*. The results from this research may help us to develop new directions and strategies for disease management in aquaculture.

**Conference Session Designation:** ( Immunology General )
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**Student Presentation:** ( Yes )

**Changes in Skin Mucus Protein Profile and Immune-Related Gene Expression in Skin of Japanese Flounder *Paralichthys Olivaceus* Fed a Diet Supplemented with High Concentrations of Ascorbic Acid**

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Ascorbic acid (AsA), commonly known as vitamin C, is an essential vitamin for normal growth and physiological function in fish. This dietary material is known to be beneficial for immune responses in fish, and increased disease resistance has been demonstrated in many fish species fed elevated levels of AsA. However, detailed knowledge concerning the beneficial effects of AsA supplementation using high concentrations of AsA with regard to the innate immune system of mucosal tissue remains to be determined. Therefore, the present study employed two-dimensional gel electrophoresis (2D-PAGE) to examine skin mucus of Japanese flounder *Paralichthys olivaceus* fed commercial diets supplemented with 2,000 mg AsA/kg diet (AsA2000) for 7 days. Compared to control fish fed a diet without AsA (AsA0), five factors were specifically detected from those fed AsA2000 and as identified by LC-MS/MS. We also performed quantitative reverse transcriptase PCR (qRT-PCR) of factors detected by 2D-PAGE and general immune-related genes from skin samples of AsA0 and AsA2000 fish. As a result, a factor with antibacterial activity was identified which was significantly up-regulated only following AsA supplementation. In conclusion, this research presents protein composition profiles of skin mucus and provides information demonstrating improvement of the mucosal immune system following high-concentration AsA supplementation in fish.

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**Live Attenuated *Edwardsiella ictaluri* Vaccines Stimulate Active Phagocytosis and Bacterial Killing of Catfish B Cells**

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*Edwardsiella ictaluri* is a Gram-negative, facultative intracellular pathogen and the causative agent of enteric septicemia of catfish, a devastating disease of farmed channel catfish. B-1 and marginal zone B cells in mammals have phagocytic abilities and contribute to innate immune responses. The innate roles of B cells have been demonstrated in several teleost fish including zebrafish, rainbow trout, and channel catfish. Recently, our group developed two protective *E. ictaluri* live attenuated vaccines (LAVs). However, their interaction with catfish B cells has not been evaluated yet. In this study, we assessed the effects of *E. ictaluri* LAVs on B cells' survival, phagocytosis, and microbial killing by flow cytometry. Results indicated that *E. ictaluri* wild-type (*Ei*WT) opsonized with sera from vaccinated and non-vaccinated catfish were engulfed by catfish B cells. However, *Ei*WT opsonized with serum from catfish survived from *Ei*WT challenge significantly decreased the numbers of B cells compared to *Ei*WT opsonized with serum from vaccinated catfish. Furthermore, catfish B cells killed *Ei*WT opsonized with sera from vaccinated catfish more efficiently compared to *Ei*WT opsonized with normal serum. Finally, we demonstrated that *Ei*WT and two LAVs induced early and late apoptotic changes in catfish B cells. These results indicate that *E. ictaluri* LAVs were enhanced active phagocytosis and killing of internalized bacteria in catfish B cells.

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