DAFINET WORKSHOP

PH.D. COURSE (4 ECTS)

FISH IMMUNOLOGY: FROM EGG TO ADULT FISH

NOVEMBER 12TH TO 14TH, 2013

Venue:

University of Copenhagen
Frederiksberg Campus
Lecture hall A2-70.04 (3-13)
Thorvaldsensvej 40
1871 Frederiksberg C
Denmark

In association with:

SCOFDA
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University of Copenhagen
Frederiksberg, Denmark

Book of abstracts

DAFINET Workshop, November 2013
University of Copenhagen

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Program

Tuesday November 12th, 2013

10.00 DAFINET Board meeting. Only for board members and external advisors

Scientific program

13:00 Welcome address by DAFINET leader Kurt Buchmann

13:15 Invited lecture by research leader Dr. Scott LaPatra,
Clear Springs Foods Inc., Research Division, Buhl, Idaho, USA
Identification of O-antigen biosynthetic genes specific to serovar 01 Yersinia ruckeri and their role in virulence and protective immunity

14:00 Coffee and tea break

14:30 Invited lecture by professor Dr. Chris Secombes
School of Biological Sciences, University of Aberdeen, Scotland
Bioactivity analysis of rainbow trout rIL-4/13, with a focus on DC-SIGN expressing cells

15:15 Postdoc Rasmus D. Heinecke
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Ontogenetic development of the trout immune system - from fertilization via yolksac larva to fry and fingerling

16:00 Postdoc Ole S. Møller
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Scanning electron microscopy techniques in fish immunology research (A simple and low-toxic method of preparing small specimens of bacteria, flagellates and their likes for Scanning Electron Microscopy)

16:15 Invited lecture by Dr. Niels Lorenzen,
Department of Animal Science, University of Aarhus, Denmark:
Recent advances in DNA vaccines for fish. B-lymphocytes are strongly involved in the inflammatory response to intramuscular DNA vaccination of rainbow trout with a plasmid encoding the VHS virus glycoprotein

16:45 Final discussions and conclusions of the first DAFINET workshop day

18:00 Dinner buffet at Stigbøjlen 7, DK-1870 Frederiksberg C (Seminar room B)

Wednesday November 13th, 2013

10:00 Invited lecture by Dr. Uwe Fischer
Friedrich-Loeffler Institute, Insel-Riems, Greifswald, Germany
Cellular immunity of rainbow trout

10:45 Postdoc Jiwan Kumar Chettri
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Environmental factors affecting challenge success in vaccination studies

11:15 Professor Niels Jørgen Olesen
National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark
Eradication of viral haemorrhagic septicaemia in Danish aquaculture

11:45 Postdoc Qusay M. Bahlool
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Immune reactions in rainbow trout against Anisakis simplex nematode larvae and their E/S antigens

12:00 Lunch at Stigbøjlen 7, DK-1870 Frederiksberg C (seminar room B)

13:00 Invited lecture by Dr. Ole Torrissen
Institute of Marine Research, Bergen, Norway
Salmon lice impacts on Norwegian salmon populations

13:30 Coffee break

14:00 Invited lecture by Dr. Bertrand Collet, Marine Scotland, Aberdeen, Scotland
SALMOCELL: Novel approaches to immortalise salmon cells

14:45 Postdoc Louise von Gersdorff Jørgensen,
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Vaccination of fish against white spot disease: New approaches

15:15 Research assistant Foojan Mehrdana
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Zoonotic metacercariae imported with ornamental fish: Risk of establishment in new geographic areas and human health aspects

15:45 Final Discussion of the second workshop day

Thursday November 14th, 2013

10:00 Invited lecture by Dr. Simon Jones,
Pacific Biological station, Nanaimo, British Columbia, Canada
Living with the enemy: responses of salmon to the parasitic copepod Lepeophtheirus salmonis include elements of immunity and tolerance

10:45 Ph.D. student Jacob Schmidt,
National Food Institute, Technical University of Denmark, Lyngby, Denmark
Expression of immune-related genes during wound healing in fish

11:15 Ph.D. student Maya M. M. Henriksen,
National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark
The immune response of rainbow trout to Flavobacterium psychrophilum following immersion-challenge model with and without hydrogen peroxide pre-treatment

11:30 Researcher Katherine Lester
Marine Scotland, Aberdeen, Scotland
Functional characterisation of the non-structural protein 2 (nsp2) of the salmon alphavirus (SAV)

12:00 Lunch at Stigbøjlen 7, DK-1870 Frederiksberg C (Seminar room B)

13:00 Research scientist Jesper Skou Rasmussen,
Department of Animal Science, University of Aarhus, Denmark
Use of DNA vaccination for determination of onset of adaptive immunity in rainbow trout fry

13:30 Ph.D. student Simon Haarder
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Increasing Contracaecum osculatum infections of Baltic cod Gadus morhua associated with grey seal colonization of spawning grounds

14:00 Postdoc Jakob Skov
Laboratory of Aquatic Pathobiology, University of Copenhagen, Frederiksberg, Denmark
Beta-glucans in fish feed – is it worth the efforts?

14:30 Final discussions and conclusions from the DAFINET workshop:
DAFINET leader Kurt Buchmann.

Abstracts

Identification of O-antigen biosynthetic genes specific to serovar 01 Yersinia ruckeri and their role in virulence and protective immunity

LaPatra S.E. [1], Welch T.J. [2]

[1]*Clear Springs Foods, Inc., Research Division, Buhl, Idaho USA*

[2]*National Center for Cool and Cold Water Aquaculture, Kearneysville, West Virginia, USA*

Yersinia ruckeri is the etiologic agent of enteric redmouth disease (ERM), a hemorrhagic septicemia that predominantly affects farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Y. ruckeri* strains comprise several O-serotypes; however the vast majority of outbreaks in farmed rainbow trout are caused by serotype O1 isolates of this pathogen. In this study we used genome sequencing to identify a large cluster of O-antigen biosynthetic genes specific to serotype O1 *Y. ruckeri* strains.

This cluster primarily consisted of genes encoding proteins predicted to function in O-antigen and LPS biosynthesis and included proteins predicted to function in the biosynthesis of Legionamic acid, a nonulosonic acid know to be part of the O-polysaccharide repeat of O1 *Y. ruckeri*. Mutation of one of the identified nonulosonic acid biosynthesis genes (*nab2*) resulted in loss of both LPS synthesis and cross reactivity with a commercially available anti-O1 serotyping antibody. This loss of LPS biosynthesis was also shown to cause a dramatic reduction in serum resistance and a complete loss of virulence in a rainbow trout challenge model. Vaccination with bacterin vaccines derived from the *nab2* mutant and its wild type parent strain was done in rainbow trout and susceptibility to *Y. ruckeri* was evaluated at various intervals post-vaccination. Significant protection against challenge with *Y. ruckeri* was only observed in fish vaccinated with the wild type strain indicating that the presence of LPS is required to mount a protective response against *Y. ruckeri* challenge. Additional vaccination experiments utilizing purified *Y. ruckeri* LPS demonstrated that LPS alone is sufficient for induction of a protective response.

Presenting Author: Scott LaPatra, scott.lapatra@clearsprings.com

Bioactivity analysis of rainbow trout rIL-4/13, with a focus on DC-SIGN expressing cells

Johansson, P.[1], Zou, J.[1], Wang, T.[1], Collet, B.[2], Secombes, C.J.[1]

[1]*Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, UK*

[2]*Marine Scotland Science, Marine Laboratory, Aberdeen, UK*

In the present study we have cloned the trout IL-4/13 paralogues, and produced the rIL-4/13A and rIL-4/13B proteins. Initial gene expression analysis confirmed that the rIL-14/13 proteins were bioactive and had similar effects, although some differences between the molecules were also apparent. In mammals IL-4 can induce the differentiation of dendritic cells, associated with an up-regulation of DC-SIGN (CD209). Thus we also examined the effect of rIL-4/13A on DC-SIGN expressing cells. Initially we cloned the trout DC-SIGN gene, and then produced a polyclonal antiserum to this molecule. DC-SIGN expressing cells represent ~3% of blood, kidney and spleen leukocyte suspensions. Stimulation of adherent head kidney cells with rIL-4/13A increased the number of DC-SIGN positive cells from ~9% to ~17% over a 120h time course study. Addition of LPS to such IL-4/13A treated cells increased the number of DC-SIGN expressing cells further, to ~28% by 24h. These results will be discussed in the context of the role of IL-4/13 in fish immune responses, and the use of markers such as DC-SIGN to help identify dendritic cells in fish.

Presenting Author: Chris Secombes, c.secombes@abdn.ac.uk

Ontogenetic development of the rainbow trout immune system - from fertilization via yolk sac larva to fry and fingerling

Heinecke, R. D., Buchmann, K.

[1]*Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark*

The rainbow trout is a cold water fish species and it produces some of the largest eggs among teleosts and has one of the longest developmental periods inside the egg before hatching. In Danish aquaculture, rainbow trout is the number one farmed fish species and infection with the protozoan skin parasite *Ichthyophthirius multifiliis* is a frequent disease. Water treatment and management of the disease causes serious impact on the environment and the economy of the farmers. Knowledge on the ontogenetic development of the rainbow trout immune system and early responses of the yolksac larvae during infection with *I. multifiliis* will improve our chances of designing and developing preventive vaccine strategies.

Using quantitative real time PCR the onset of expression of different immunologically related molecules during the ontogenetic development of the rainbow trout from fertilization to the early post-hatch larvae was studied. Immunohistochemistry was used to localize the expression of five different immune relevant protein molecules during development. Further, we examined the ability of young post-hatch larvae to regulate different cytokines, chemokines and acute phase proteins in response to infection with the skin parasite *Ichthyophthirius multifiliis*.

The expression of the different immune molecules in the rainbow trout embryo was initiated early after fertilization but at very low levels during the egg phase and expression generally increased around the time of hatching. In the post-hatch larva immunohistochemical staining was seen for CD8 in the thymus, IgT in gill mucus, MHCII in thymus, intestine, skin, gills and pseudobranch. Heavy staining was seen for SAA in the pseudobranch. The early post-hatch larvae infected with *I. multifiliis* were able to respond immunologically by differentially expressing and regulating a number of immune genes. These studies reveal that the ontogenetic development of the rainbow trout immune system was initiated at an early time point after fertilization of the egg and the early post-hatch larvae were able to mount an inflammatory response upon infection with the parasite *I. multifiliis*.

Presenting author: Rasmus Demuth Heinecke, rdh@sund.ku.dk

A simple and low-toxic method of preparing small specimens of bacteria, flagellates and their likes for Scanning Electron Microscopy

Møller O.S.[1], Buchmann K.[1], Dalsgaard I.[2]

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[2]*National Veterinary Institute, Section for Bacteriology, Pathology, and Parasitology, Technical University of Denmark, Denmark*

The preparation of samples of bacteria and other very small organisms (<50 µm) for Scanning Electron Microscopy is often complex and intricate, which typically involve the use of specialized filter systems, complex handling and toxic chemicals. Based on the methods described in the literature and our own tests, we have distilled a simpler (although slightly crude) method to prepare bacterial samples in a fast way. We only employ readily available chemicals requiring no more than a fume hood, and low-cost, standard lab equipment like single use filters. The method is excellent for achieving relatively quick results for illustration purposes and does not require handling of highly toxic substances like Osmium-tetraoxide, which typically necessitates skilled/trained lab personnel. Thus, this method is well-suited for testing different bacterial concentrations, biotypes, and other variables relatively quickly. So far, this method has yielded good results on several pathogenic bacteria and parasites; *Aeromonas salmonicida*, *Yersinia ruckeri*, *Ichthyobodo necator* and theronts of *Ichthyophthirius multifiliis*.

Presenting author: Ole Steen Møller, Oles@sund.ku.dk

B-lymphocytes are strongly involved in the inflammatory response to intramuscular DNA vaccination of rainbow trout with a plasmid encoding the VHS virus glycoprotein

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[4]*University of Southern Denmark, Odense, Denmark*

DNA vaccines encoding rhabdovirus glycoproteins provide high protection when delivered to salmonid fish by intramuscular injection of naked plasmid DNA. The local reaction in the injection site is characterized by a relatively fast infiltration by leucocytes which presumably play a role in establishment of protection. The current study aimed at characterization of these infiltrating cells.

Monoclonal antibodies generated against recombinant rainbow trout IgM and IgT heavy chain protein fragments were applied in immunohistochemical staining of muscle tissue sections from DNA vaccinated rainbow trout fingerlings. A strong staining for both IgM and IgT was observed, but with different kinetics. At 7 days post vaccination at 15C, IgM-positive cells were dominating whereas IgT-positive cells were most prevalent at 21 days post vaccination. Although functional aspects remain to be determined, it is interesting that the two phase scenario seems to timely correlate with the change of the nature of the vaccine induced protective mechanisms from first being innate to later becoming adaptive. Altogether the results suggest that B-cells could play other roles than antibody production in establishment of protective immunity in rainbow trout.

Presenting author: Niels Lorenzen, Niels.Lorenzen@agrsci.dk

Cellular immunity in fish:

Characterization of cells involved in adaptive immune responses in fish

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The classical mammalian nomenclature subdivides T cells into several subpopulations of which T helper (Th) cells and cytotoxic T lymphocytes are the most important. However, many studies in fish still lack to demonstrate their cellular and functional equivalents.

Th1 and Th2, the major subdivisions of T helper cells, were first demonstrated in mice by analysing clonal T cell lines. To identify Th subsets in fish, we developed co-culturing systems of carp leukocytes with supporting cells resulting in long-term proliferation of Th-like cells. Moreover, we succeeded to clone Th cells from these bulk-cultured T cells. One clone was established as a long-term cell culture and shown to express the Th2-master regulator GATA-3 but not the Th1-master regulator T-bet. PHA stimulation triggered the transcription of the Th2-related cytokine IL-4/13B, but not of the Th1-related cytokine IFNγ. These results indicate that this clone represents a Th2-like subset which is hallmarked by *GATA-3* and *IL-4/13B* expression.

The T-box transcription factor Eomesodermin (Eomes) is associated with function and differentiation of NK and $CD8^+$ T cells. We identified two teleost *Eomes* genes (*-a* and *-b*) in rainbow trout and ginbuna carp, both containing highly conserved T-box DNA binding domains. While their high abundance in brain and ovary is probably not associated with immune functions their expression in lymphoid tissues presumably is. Investigation of pronephrocyte subpopulations indicated that both transcripts were few or absent in IgM^+ lymphocytes, but more abundant in IgM^- /$CD8\alpha^+$ and IgM^-/$CD8\alpha^-$ populations. Expression analysis of sorted $CD8\alpha^+$ lymphocytes from trout mucosal and non-mucosal lymphoid tissues demonstrated that the distribution of *Eomes-a/b*, *T-bet*, and *Runx3* versus *IFN-γ* does not reveal simple correlations, suggesting tissue-specificity of $CD8\alpha^+$ lymphocytes and/or modes that drive IFN-γ expressions.

Presenting Authors:
Uwe Fischer, Uwe.Fischer@fli.bund.de

Environmental factors affecting challenge success in vaccination studies

Chettri, J. K.[1], Skov, J.[1], Dalsgaard, I.[2], Buchmann, K.[1]

[1]*Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark*

[2]*National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark*

We have tested the efficacies of two different vaccines (a commercial versus an experimental vaccine – both being oil adjuvanted) for rainbow trout against furunculosis caused by *Aeromonas salmonicida* infections. However, when challenging fish with live bacteria in order to assess protection following vaccination, the administration of the pathogen is important for the outcome of the experiments. We have therefore also compared injection challenge with cohabitation challenge. In addition, when doing so we also investigated the influence of environmental conditions such as salinity and temperature on the protection recorded. Thus, challenge studies were conducted at two temperatures (12 and 19°C) and at two salinities (0 and 15 ppt). Mortalities following challenge were recorded and RPS calculated for each group. Side effects of the vaccines were evaluated by using the Speilberg scale. Results from the challenge studies will be presented.

Presenting author: Jiwan Kumar Chettri, jkc@sund.ku.dk

Eradication of viral haemorrhagic septicaemia in Danish aquaculture

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[3] *Danish Aquaculture Association, Silkeborg, Denmark*
[4] *Danish Veterinary and Food Administration, Glostrup, Denmark*
[5] *Danish Veterinary and Food Administration, Vejle, Denmark*

Viral haemorrhagic septicaemia (VHS) virus was first isolated in Denmark in 1962, when more than half of the approximately 800 Danish fish farms were considered to be infected. Today, 50 years later, the country obtained status as EU approved VHS free zone. In the years in between very significant resources have been used to control and eradicate the disease.

The control program included strict biosecurity and preventative measures, trade regulations, zoning and intensive inspections and laboratory testing. During the first decades of control and eradication programs the number of infected farms was significantly reduced while the curve flattened the last 20 years. It was only after a large and costly coordinated action in 2009-2013 including all affected areas that the country managed to free itself totally from VHS.

Molecular tracing of the origin of VHSV isolates revealed that despite strict trade regulations and ban on introduction of live salmonids into the country VHSV seemed to have crossed the boarders into Denmark in a couple of cases.

It is the first time that VHS has been eradicated from an endemically infected country. Among the causes of the success are a close collaboration between industry, stakeholders, veterinary authorities and scientists. Also the reduction of the number of farms and novel farming strategies account for the success. Furthermore, in Denmark rainbow trout farming would not survive in the international competition being endemically infected with this serious disease providing a strong incitement for the fish farmers.

Vaccination was not included in the control in Denmark but if licensed, vaccines would have been useful in order to reduce virus load before stamping-out. Similar control strategies will hopefully be implemented in other VHS infected countries in order to improve fish health and efficiently combat the disease.

Presenting author: Niels Jørgen Olesen, njol@vet.dtu.dk

Immune reactions in rainbow trout against *Anisakis simplex* nematode larvae and their E/S antigens

Bahlool Q.Z.M, Kania P.W., Skovgaard A., Haarder S., Buchmann K.

Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark

Third stage larvae of *Anisakis simplex* (Nematoda: Anisakidae) are capable of infecting a wide range of fish species including salmonids, which serves as paratenic hosts for the nematode. Nevertheless, scarce knowledge is available concerning the defence mechanisms initiated by *Anisakis* infection in fish. We conducted several *in vivo* studies in rainbow trout (*Oncorhynchus mykiss*) in order to understand more aspects of *Anisakis*-fish relationship and the immune reactions against this nematode infection. An additional experiment was also conducted to elucidate the influence of excretory/secretory (ES) products from *Anisakis simplex* on the immune system of rainbow trout. Results of the different performed experiments will be presented and discussed.

Presenting author: Qusay Bahlool, qusay@sund.ku.dk

Salmon lice impacts on Norwegian salmon populations

Torrissen O.

Institute of Marine Research, Bergen, Norway

A status report on infections with salmon lice affecting wild and cultured Atlantic salmon (*Salmo salar L.*) in Norwegian waters will be presented. Possible threats and future solutions will be discussed.

Presenting author: Ole Torrissen, ole.torrissen@imr.no

SALMOCELL: Novel approaches to immortalise salmon cells

Collet B., Collins C.

Aquaculture and Marine Environment, Marine Scotland, UK

There is currently a lack of well characterised Atlantic salmon cell lines available for research and for diagnostics. The few cell lines available have been isolated empirically and are poorly characterised. As a result, most of the research on fish infectious diseases depends directly on experimental animal infections in contained facilities. The access to specific cell types corresponding to tissues showing strong viral tropism such as gill epithelial cells for Infectious Salmon Anaemia Virus or cardiomyocytes for Salmon Alphaviruses, or having specific functions such as immune cell types, would allow wider application of *in vitro* research on important fish diseases, ultimately reducing the number of *in vivo* studies.

In this new project SALMOCELL, funded through the Pilot Grant scheme of the UK National Centre for the Replacement, Refinement, and Reduction of animals in research (NC3Rs), we propose to explore innovative approaches for the generation of novel *Salmo salar* cell lines. Firstly, we aim to attempt induction of pluripotency in established cells lines such as the Atlantic Salmon Kidney (ASK) cell line in order to generate a stem cell line that can be further re-programmed into any cellular type required. Secondly we plan to use oncogenes, genes with anti-apoptotic properties or genes expressing growth factors to generate cells lines from primary cultures. Specific genes will be over-expressed by transgenesis. Cells from salmon eggs, embryo or juveniles will be transfected immediately after dissociation to retain a maximal diversity of cell types. Plasmids expressing one or several genes will be used and stable transfectants will be cloned and characterised further for their propagation property and phenotypes. A large set of expression plasmids containing relevant genes have already been constructed or obtained from different sources and will be tested shortly on fish cells obtained from salmon early life stages.

Presenting Author: Bertrand Collet, Bertrand.Collet@scotland.gsi.gov.uk

Vaccination of fish against white spot disease:

Yersinia ruckeri as a vector for an anti-parasitic vaccine in fish

Jørgensen L.vG., Kania P.W., Buchmann K.

[1]*Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark*

Ichthyophthirius multifiliis (Ich) is a parasite, which causes white spot disease in freshwater fish. It is a major problem for the aquaculture industry and commercial vaccines are not yet available. A protein on the parasite surface called the immobilization antigen (I-ag) has been the major focus for vaccine development in recent years. DNA vaccines encoding this protein have been tested but did not provide any protection. The purified protein as a vaccine has also been evaluated, however a protection level was only seen when adjuvants were applied. This project will combine the use of the I-ag as a vaccine against Ich with an available vaccine against enteric red mouth disease, caused by the bacterium *Yersinia ruckeri* (Yr). Yr will function as the transporter and the adjuvant for the I-ags. The bacterium will be genetically modified in such a way that it expresses the I-ag. The idea is to make a bacterin with the gene modified *Yersinia*. Rainbow trout will be immersed for 30 seconds in the bacterin and any subsequent protection against white spot disease and/or enteric red mouth disease will be evaluated.

Another part of this project includes an investigation of where the immunological response against the parasite begins. The aim is to elucidate in which organs and locations parasite antigens are processed in a zebrafish model. The transparent mutant zebrafish *Casper* will be used. Parasite antigens will be coupled to the fluorescence marker GFP and a gene modified *Yersinia ruckeri* expressing the parasite antigen and GFP will be constructed. From this GMO a bacterin will be made. *Casper* will be immersed into the bacterin for 1 minute to 1 hour and a subsequent detection of GFP and antigen will be conducted by fluorescence imaging and immunohistochemistry.

Thanks to Karina Juhl Rasmussen, Karsten Skjødt, Marianne Halberg Larsen and Dorte Frees for their help so far…

Presenting author: Louise von Gersdorff Jørgensen, lvgj@sun.ku.dk

Zoonotic metacercariae imported with ornamental fish: Risk of establishment in new geographic areas and human health aspects

Mehrdana F., Jensen H.M., Kania P.W., Buchmann K

Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark

Fish-borne zoonotic trematodes are considered as a serious health problem in countries where ingestion of raw or undercooked fish products is common. This tradition has recently been introduced in the industrialized part of the world including Europe. It is therefore relevant to report a recent introduction of ornamental fish, *Xiphophorus maculatus*, heavily infected with exotic metacercariae of *Centrocestus* sp. into Denmark. A Danish company, which imported a batch of fish from Singapore, complained about lethargic and morbid fish despite several attempts of treatment for common diseases. We subjected a number of the fish to a full parasitological examination and found heavy infections with *Centrocestus* sp. metacercariae (100% prevalence). Diagnosis was performed using molecular and classical parasitological techniques. The risk of spread of this and related diseases in European waters is discussed in line with the veterinary procedures for border inspection of imported exotic fish species.

Presenting Author: Foojan Mehrdana, Foojan@sund.ku.dk

Living with the enemy: responses of salmon to the parasitic copepod *Lepeophtheirus salmonis* include elements of immunity and tolerance

Jones S.R.M.[1], Sutherland B.J.G.[2], Braden L.M[2], Koop B.F.[2]

[1]*Pacific Biological Station, Nanaimo, British Columbia, Canada*

[2]*Centre for Biomedical Research, Department of Biology, University of Victoria, Canada*

Susceptibility to infection with the parasitic copepod *Lepeophtheirus salmonis* is remarkably diverse, resulting in a broad range of disease consequences among host species. Pink and coho salmon are relatively resistant whereas chum and sockeye salmon are susceptible and the earlier research indicated that resistance was associated with a rapid and robust inflammatory response in the skin. In new experiments, parasitological, physiological and transcriptomic data were collected to explore explanatory mechanisms for infections in pink, chum and Atlantic salmon.

Size-matched salmon belonging to each species were cohabited and all fish in a tank were sampled between 3 and 43 days post exposure. Consistently higher levels of infection were observed on chum, followed by Atlantic and pink salmon and these were associated with elevated plasma cortisol in chum salmon and reduced haematocrits in chum and Atlantic salmon. Gene expression in head kidney and skin measured using a 4x44k Agilent oligo-array and by real-time PCR, revealed that infection influenced response pathways in all species and included changes in the acute phase response, complement and coagulation, stress and anti-viral suppression. Coincidentally, pathways associated with tissue remodelling, antioxidant activity and unfolded protein responses suggested tolerance to the negative consequences of infection is adaptive. In susceptible salmon, pathways of antigen presentation were depressed whereas in resistant salmon, iron homeostasis, innate pattern recognition receptors and markers of inflammation were upregulated. The presence of defense- and tolerance-associated responses during infection with *L. salmonis* suggests that optimising bioenergetic expenditures while coping with infection is adaptive in salmon.

Presenting Author: Simon Jones, simon.jones@dfo-mpo.gc.ca

Expression of immune-related genes during wound healing in fish

Schmidt J.G., Nielsen M.E.

National Food Institute, Biological Quality Research Group,
Technical University of Denmark, Søborg, Denmark

Sterile tissue damage triggers the same pathways through damage-associated molecular patterns (DAMPs) that an infection does through pathogen-associated molecular patterns (PAMPs), albeit with different kinetics of expression of the involved genes. The cascade of reactions initiated by tissue damage starts with a series of non-transcriptional responses that leads to vasoconstriction and hemostasis. This is usually followed by an inflammatory response also initiated in the absence of transcription, but later greatly enhanced by expression of genes coding for proinflammatory cytokines and proteases. Inflammation is aimed at eradicating invading pathogens but causes concomitant tissue damage and this compromises regeneration. A strong inflammatory response often results in fibrosis and poor repair of the wound site, and is thus disadvantageous if the wound is uninfected. However, the inflammatory response is limited in early life stages of most animals, and these heal with a much higher degree of regeneration.

I will be presenting a selection of results from my PhD studies with a focus on the response to wounding during ontogeny of carp larvae and juveniles aged 7-56 days post-fertilization. The wounds of larvae completely regenerate within 3 days, whereas the wounds in juveniles are still visible 7 days post-wounding. Wound-induced changes in gene expression are limited, which may be ascribed to similarities between natural morphogenesis and tissue repair and that the investigated genes are thus already transcribed at adequate levels. However, expression of the mucosal immunoglobulin IgZ1 is upregulated in wounded larvae, indicating important innate effector functions of this isoform during early ontogeny.

Presenting author: Jacob Schmidt, jacsc@food.dtu.dk

The immune response of rainbow trout to *Flavobacterium psychrophilum* following immersion-challenge model with and without hydrogen peroxide pre-treatment

Henriksen M.M.M.[1], Madsen L.[1], Kania P.W.[2], Buchmann K.[2], Dalsgaard I.[1]

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The bacterial fish pathogen *Flavobacterium psychrophilum* is a major cause of mortality in farmed rainbow trout (*Oncorhynchus mykiss*) and other salmonid fish. The disease following infection is called bacterial coldwater disease (BCWD) or rainbow trout fry syndrome (RTFS). To our knowledge, no commercial vaccine is currently available and the disease is treated with antibiotics. Injection-based challenges with *F. psychrophilum* are standardized but the route of infection does not reflect a natural situation. Therefore, we established an immersion-based model investigating if hydrogen peroxide (H_2O_2) pre-treatment could elevate infection and mortality.

The model consisted of four groups: 1) Un-exposed control, 2) H_2O_2 exposure, 3) *F. psychrophilum* immersion and 4) H_2O_2 + *F. psychrophilum*. Pre-treatment with H_2O_2 increased mortality two-fold if fish also were exposed to *F. psychrophilum* after pretreatment. Tissue samples were taken from the involved groups 4 h, 48 h, 125 h and 192 h post-exposure and investigated for regulation of immune genes. Following genes were examined in the head kidney and gills by qPCR: IgT, IgM, CD8, CD4, MHC I, MHC II, IL-4/13A, TcR-β, IL-10, IL-6, IL-1β, IL-17, SAA and FoxP3.

A pro-inflammatory response was indicated, but only a weak indication of an adaptive response was recorded (most evident in the *F. psychrophilum* group). Further, pre-treatment with H_2O_2 affected the correlation gene expression and pathogen load in several cases. Morphological changes in the gill tissue were evaluated using hematoxylin and eosin stained tissue sections. Exposure to both H_2O_2 and *F. psychrophilum* intensified tissue damage and postponed healing. The results indicate that *F. psychrophilum* may have an immunosuppressive action and that environmental stress may be one of several factors playing a role in RTFS outbreaks.

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Functional characterisation of the non-structural protein 2 (nsp2) of the salmon alphavirus (SAV)

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Pancreas Disease (PD) is one of the most significant viral diseases of salmonids aquaculture in Scotland and worldwide, and is responsible for considerable economic loss. In Atlantic salmon, it was first described in Scotland in 1976 and is characterised histopathologically by pancreatic and cardiomyocytic necrosis and skeletal myopathy. Outbreaks are associated with variable mortalities ranging from 0.1-63% in the period of 1989 to 1994 in Ireland; however survivors often fail to reach optimal weight and have to be graded out as runts. Salmonid alphavirus (SAV), identified as the viral agent of PD and belonging to the family *Togaviridae*, has a single stranded positive sense RNA genome of approximately 12 Kb. The 3' third comprises the structural proteins, including the capsid, E3, E2, 6K and E1 and is polyadenylated whilst the 5' two thirds code for the non-structural proteins nsP1-nsP4. There are three untranslated regions, one at the 5' end, one between nsP4 and the capsid and another at the 3'end followed by a poly A tail.

In alphaviruses infecting other vertebrates, nsP2 has been described as a multifunctional protein with proteinase activity in the C-terminal domain, termination cessation of minus strand synthesis, correct folding of the nonstructural proteins and interferon inhibitor.

Very little is known about the function of the SAV nsP2. Two subtypes 1 SAV isolates, a cell culture adapted F93-125 and a low passage filed isolate 4640 have very similar genome sequence but very different ability to replicate and induce interferon (IFN) in vitro. To elucidate the detailed function of SAV nsP2, two expression plasmids encoding for nsP2 isolated from SAV1 F93-125 or 4640 were constructed and used to characterise the cellular localisation and nsP2 ability to interfere with the IFN response. Stable cell lines clones over-expressing nsP2 F93-125 or nsP2 4640 under the control of doxycyclin (Tet-off system) were isolated by stable transfection of CHSE-TOF and nsP2. The permissivity of these clones to a range of fish viruses was investigated and compared with the parental CHSE-TOF cell line.

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Use of DNA vaccination for determination of onset of adaptive immunity in rainbow trout fry

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Vaccine producers often recommend a minimum size of 5g for vaccination of rainbow trout, but implementation of prophylactic vaccination in smaller sized fish would be an advantage for several infectious diseases. To implement a cost efficient vaccination strategy, it is important to know the duration and nature of the protective immunity induced by the vaccines in the fish. The present work aimed at determination of the smallest size at which specific immunity could be induced in rainbow trout fry by DNA vaccination against viral haemorrhagic septicaemia (VHS). Since the vaccine is known to induce both innate and adaptive protection, we also aimed at determination of which type of protection the DNA vaccine induced in early life stages of rainbow trout.

Vaccination trials were performed with fry at average sizes of 0.25 g and 0.5 g respectively and included both the homologous VHSV G-gene vaccine and a heterologous DNA vaccine encoding the G-protein of infectious haematopoietic necrosis virus (IHNV). The fish were challenged by immersion at different times after vaccination. Protective immunity was induced in both sizes of fish, but whereas robust specific protection was evident in the fish vaccinated at 0.5g, the results suggested that the protection in the fish vaccinated at 0.25 g was mainly due to innate cross-reactive antiviral mechanisms of shorter duration. The critical size for induction of an adaptive immune response in rainbow trout to this type of vaccination thus appears to be between 0.25 and 0.5g.

Tissue samples were taken from the fish at different time points after vaccination and during viral challenge. Quantitative RT-PCR (QPCR) was used to analyse the expression of a panel immunologically relevant genes, with the aim of correlating the observed development of immunity with the ontogeny of the fish immune system.

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Increased *Contracaecum osculatum* infection of Baltic cod (*Gadus morhua* L.) liver associated with increasing grey seal (*Halichoerus gryphus* L.) populations

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Grey seals (*Halichoerus gryphus*), the main definitive host of the anisakid nematode *Contracaecum osculatum* in the Baltic, have re-colonized the southwestern Baltic within the last decade. The economically important Baltic cod (*Gadus morhua*) serves as transport host for *C. osculatum;* thus, we hypothesized that the colonization process could be associated with an increase in prevalence and intensity of third stage larvae of *C. osculatum* in liver of Baltic cod.

We performed a parasitological study of cod in spring 2012 and compared the results with previously unpublished data from the early 1980's. Further, grey seals were counted annually from 2000 to 2011 at three haul-out sites in the southwestern Baltic. Out of the 97 cod livers examined in the 1982/1983 survey, 22% harbored *C. osculatum* larvae; the mean intensity was 4.3 and the mean abundance 0.9. In 2012 it was found that 55.1% of the examined cod livers (*n*=185) were infected; the mean intensity was 20.2 and the mean abundance 11.1. Nematode samples from 2012 were subjected to molecular methods (PCR) which confirmed the identity of the larvae. The grey seal population in the study area increased more than tenfold during the 12 year period.

The results suggest that the elevated parasitization of cod liver is associated with the successful re-establishment of grey seals in the southwestern Baltic. The potential economic impact on local fishing communities is discussed and possible management plans are presented.

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β-Glucans in fish feed – is it worth the efforts?

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A huge body of literature reports immunostimulatory effects of β-glucan, a polysaccharide of e.g. fungi, algae and bacteria, in a broad range of recipient animals including both invertebrates (e.g. crustaceans) and vertebrates (e.g. fish, mice and man). Observed immunostimulatory effects following β-glucan administration range from improved wound healing, increased resistance towards stress and infections with viruses, bacteria, fungi and parasites, to tumor regression in cancer studies. Despite the large number of studies investigating the immunostimulatory potential and actions of β-glucans, no consensus exists on the basic physico-chemical β-glucan features required for immunostimulation. Furthermore, adverse effects indicating β-glucan induced immunosuppression are rarely and insufficiently covered by literature reviews, which mainly highlight the positive effects of β-glucan administration. Difficulties in identifying optimal β-glucan dosage and administration schedules represent another problem, which require further attention and explanation. At present, there is a need to broaden and strengthen our understanding of the actions and use of β-glucan as an immunostimulant.

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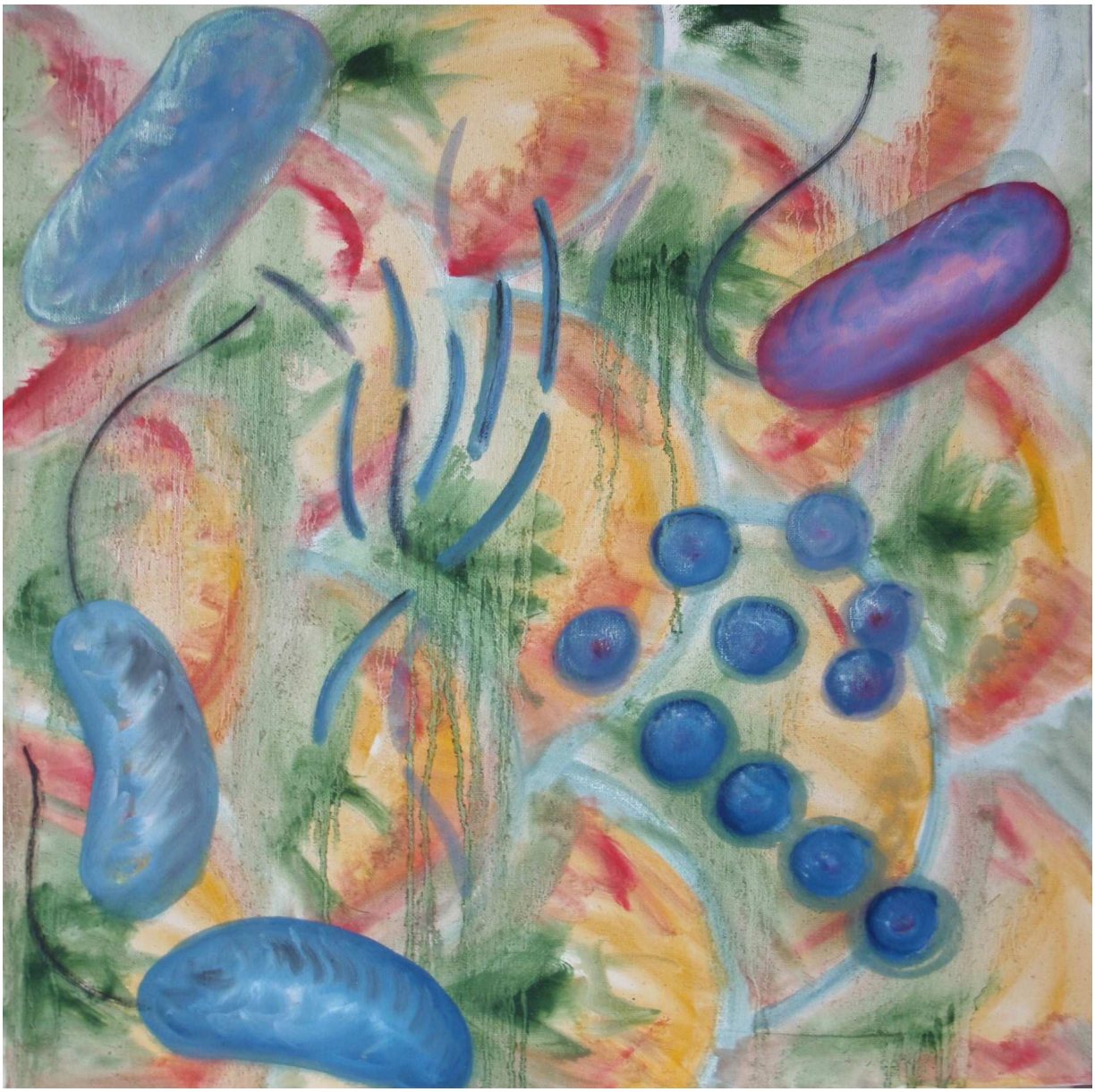

Illustration by Kurt Buchmann