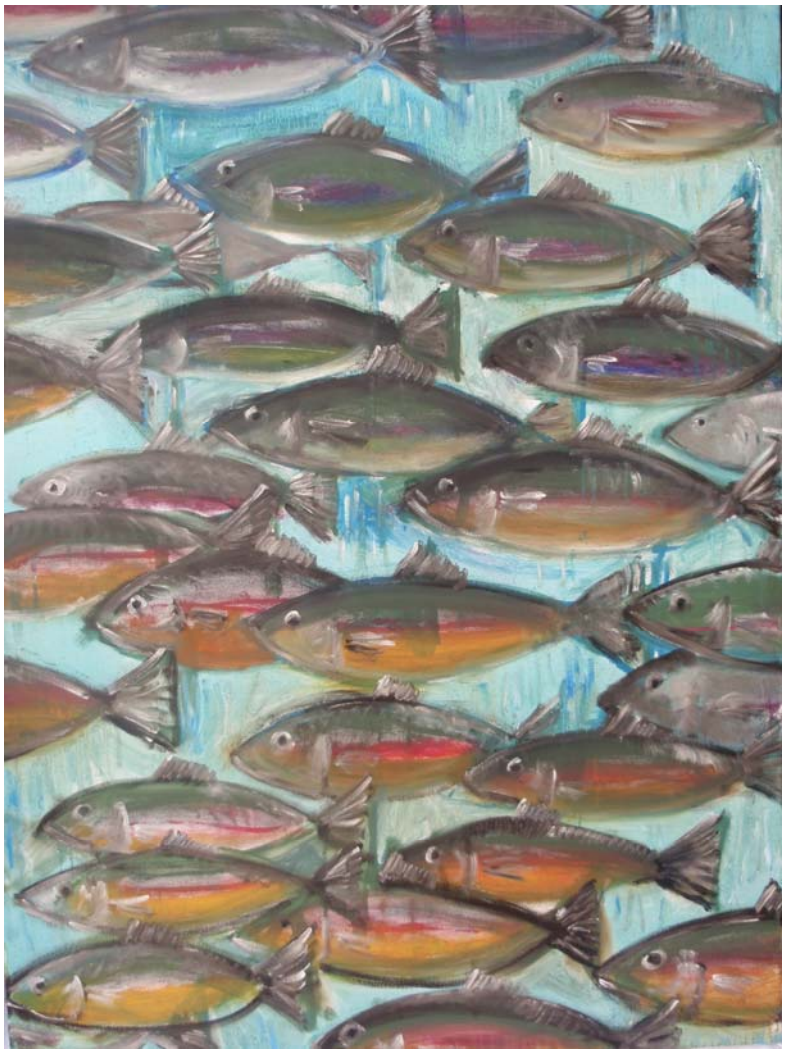
DAFINET AND TARGETFISH FP7

WORKSHOP AND PH.D. COURSE

FISH MODELS IN RESEARCH



NOVEMBER 11TH TO 13TH, 2014

Venue:

University of Copenhagen
Frederiksberg Campus
Lecture hall 2.01 (A1-04.01)
Grønnegaardsvej 7
1870 Frederiksberg C
Denmark

Organised by:

DAFINET www.dafinet.dk

The Graduate School for Immunology and Infectious Diseases, KU-SUND

TargetFish FP7

Book of abstracts

DAFINET and TargetFish FP7 Workshop, November 2014
University of Copenhagen

The workshop is supported by:

TargetFish which is a large collaborative project funded by the European Commission (Grant Agreement No. 311993) under the 7th Framework Programme for Research and Technological Development.

DAFINET, Danish Fish Immunology Network

P.C. Abildgaard Foundation

The Graduate School for Immunology and Infectious Diseases, Faculty of health and Medical Science, University of Copenhagen

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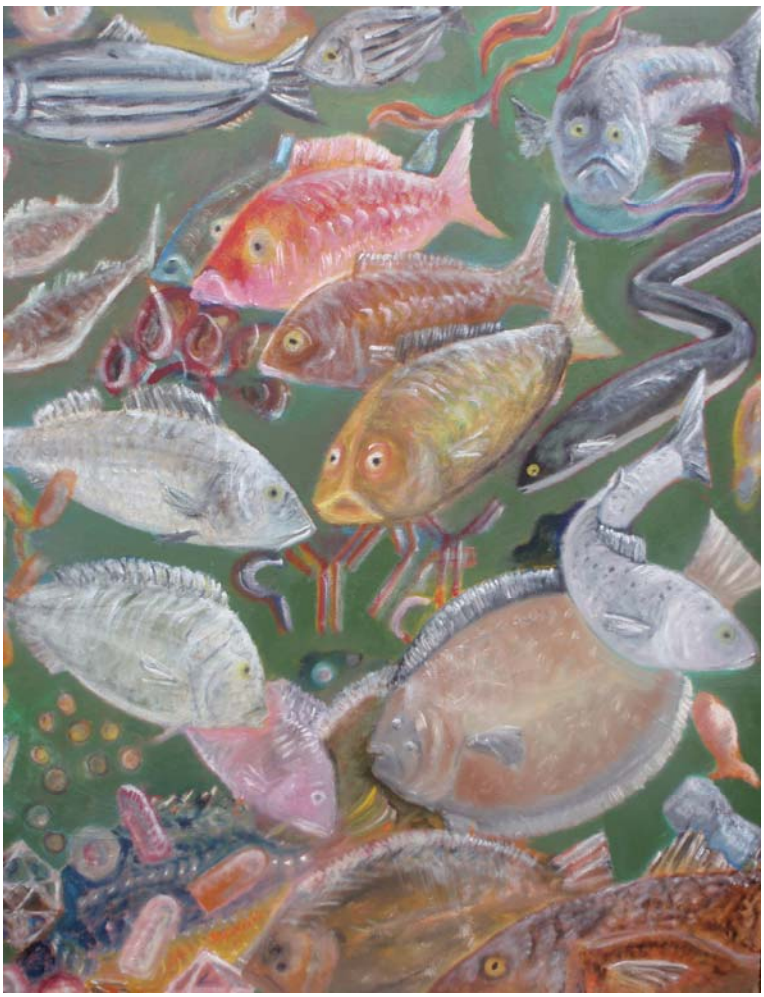

Invited speakers:

Peter	Aleström	Norway
Jane	Behrens	Denmark
Maria	Forlenza,	Netherlands
Jorge	Galindo-Villegas	Spain
Simon	Haarder	Denmark
Louise	Jørgensen	Denmark
Scott	LaPatra,	USA
Niels	Lorenzen	Denmark
Barbara	Nowak	Australia
Elke	Ober	Denmark
Miguel	Rubio-Godoy	Mexico
Guiseppe	Scapigliati	Italy
Sune	Sørensen	Denmark
Jonna	Tomkiewicz	Denmark

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TargetFish FP7

Welcome address and introductory remarks

K. Buchmann, University of Copenhagen, Denmark

The Danish Fish Immunology Research Center and Network (www.dafinet.dk) was supported by the Danish Strategic Research Council from 2009 to 2013. Due to the exciting results and stimulating discussions obtained during these five years we decided from 2014 to continue some of the activities now in collaboration with the graduate school of infectious diseases and immunology at the Faculty of Health and Medical Sciences. We carry on by organizing annual workshops in collaboration with TargetFish which is a large collaborative project funded by the European Commission (Grant Agreement No. 311993) under the 7th Framework Programme for Research and Technological Development. The workshop is also supported by the Abildgaard Foundation established in the memory of Dr. Peter Christian Abildgaard, the founder (in 1773) of the first veterinary school in Denmark and Scandinavia. This excellent scientist was not only a brilliant fish parasitologist but was also the first to carry out experimental infections when elucidating (in 1789) the life cycle of the fish tapeworm *Schistocephalus solidus.* There is direct line from these early studies to the workshop of today – elucidating how fish can be used as experimental models. Various fish species are gaining increasingly higher importance worldwide, not only within aquaculture production, but also within biological, molecular and biomedical research. The use of fish as experimental animals and models has outnumbered the use of mammals such as mice, rats and rabbits in certain countries such as Norway where far more than 1 mio fish are being used for this purpose each year. Prominent classical species are Atlantic salmon, rainbow trout and common carp but during the latest decades the zebrafish has obtained status as the number one experimental fish of the future. However, due to the wide variation within the fishes (which currently count around 30,000 species) with regard to physiology, immunology and anatomy it will be important in the future to conduct comparative studies within all scientific branches involving fish. It must be kept in mind that although a certain fish species can be used as model for even human functions the genetic distance between two fish species can be longer than that between carp and man. The present workshop aims at addressing some of the research areas in which fish can be used as models. By calling in excellent researchers covering more than nine fish species from various taxonomic groups we hope to stimulate new contacts, collaborations and projects taking these aspects into account.

Program

Tuesday November 11th, 2014

13:00 Professor Peter Alestrøm
Department of Basic Science Norwegian University of Life Sciences, Norway
The maternal zygotic transition (MZT) in zebrafish

13:30 Associate Professor Elke Ober
The Danish Stem Cell center, University of Copenhagen, Denmark
Liver organogenesis in zebrafish

14:00 Ph.D. student Simon Haarder
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Zebra fish as a model for inflammatory bowel disease (IBD): preliminary results

14:15 Coffee break

14:45 Research leader Dr. Scott LaPatra
Clear Springs Foods Inc., Research Division, Buhl, Idaho, USA
Biosecurity in fish keeping facilities

15:15 Postdoc Louise von Gersdorff Jørgensen
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
A search for new vaccine candidates in *Ichthyophthirius multifiliis*

15:30 Professor Niels Lorenzen
Department of Animal Science - Fish Health, Aarhus University, Denmark
Rainbow trout as a model in functional studies of anti-viral immunity

16:00 Professor Giuseppe Scapigliati
Dept. DIBAF, University of Tuscia, 01100 Viterbo, Italy
The European sea bass as a model species in immunobiology

16:30 Assistant Professor Maria Forlenza
Animal Science Group, Wageningen University, The Netherlands
Common carp as experimental animal model

17:00 Final discussions and conclusions of the first DAFINET workshop day

18:00 Welcome Dinner

Program
Wednesday November 12th, 2014

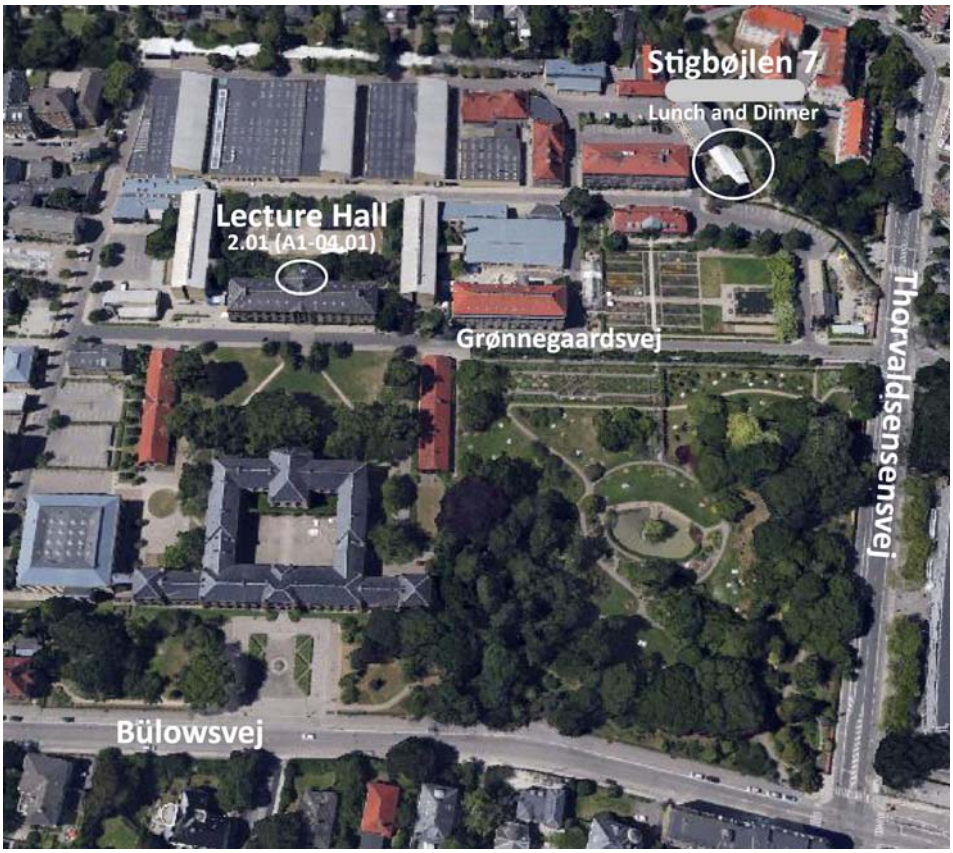
10:00 Professor Barbara Nowak
NCMCRS AMC, University of Tasmania, Tasmania
Atlantic salmon as an experimental model

10:30 Research leader Dr. Scott LaPatra
Clear Springs Foods Inc., Research Division, Buhl, Idaho, USA
Methods Used for the Evaluation of Vaccine Efficacy

11:00 Research scientist Jane Behrens
National Institute of Aquatic Resource, Technical University of Denmark
The parasitic copepod *Lernaeocera branchialis* negatively affects cardiorespiratory function in Atlantic cod (*Gadus morhua*)

11:30 Professor Barbara Nowak
IMAS, University of Tasmania, Tasmania
Tuna as an experimental model

12:00 Lunch
13:00 Excursion to the Danish national Aquarium: The Blue Planet
16:00 Back to Frederiksberg Campus
18:00 Workshop dinner



Program
Thursday November 13th, 2014

10:00 Professor Barbara Nowak
NCMCRS AMC, University of Tasmania, Tasmania
Barramundi and other Australian fish species as experimental models

10:30 Postdoc Subhodeep Sarker
Clinical Division of Fish Medicine, University of Veterinary Medicine, Austria
RNA interference (RNAi) as a possible control of whirling disease in rainbow trout (*Oncorhynchuss mykiss*)

10:50 Postdoc Jorge Galindo-Villegas
The Fish Phagocyte & Cytokine Lab, Faculty of Biology, Murcia University, Spain
Recent achievements on the evolution and diversity of the seabream immune system

11:10 Senior research scientist Jonna Tomkiewicz
National Institute of Aquatic Resource, Technical University of Denmark
European eel as experimental model I:
Assisted reproduction technology and standardized fertilization methods for mass production of viable embryos and larvae

11:40 Postdoc Sune Riis Sørensen
Section for Population Ecology and Genetics, Technical University of Denmark
Improving biophysical rearing conditions during early life stages of European eel

12:00 Lunch

13:00 Professor Miguel Rubio-Godoy
Instituto de Investigaciones Biomédicas,
Universidad Nacional Autónoma de México
Tilapia and poeciliid fishes as experimental models

13:30 Ph.D. student Hanne Haatveit
Faculty of Veterinary Medicine and Biosciences,
Norwegian University of Life Sciences, Norway
The non-structural proteins of piscine orthoreovirus, organizers of virus assembly?

13:50 Ph.D. student Jesper Kuhn
Department of Arctic and Marine Biology, University of Tromsø, Norway
Effects of fish species composition on Diphyllobothrium spp. infections in subarctic brown trout and Arctic charr
- is the three-spined stickleback a key species?

14:10 Ph.D. student Morten Lund
Norwegian Veterinary Institute, Oslo, Norway
Challenge models to clarify effects of piscine orthoreovirus (PRV) infection in blood cells on salmon robustness for oxygen deprivation, smoltification, stress and secondary infections

15:00 Final discussions and conclusions of the workshop

The maternal zygotic transition (MZT) in zebrafish

P. Aleström

Department of Basic Science Norwegian University of Life Sciences, Norway

Zebrafish (*Danio rerio*), a tropical aquarium fish from the inland water systems of North-East India and Pakistan, was initially selected as vertebrate model for embryo development because of its attributes of being a small, robust and easy to breed fish with transparent embryos which develop to hatching within 48-72 hours at 28°C. During the last 30 years a detailed knowledge about zebrafish biology and genetics has been generated and is to a large extent available to the international zebrafish community at the web site ZFIN. The well-organized access to zebrafish biology, genetics, husbandry and disease, as well as to defined wild-type strains, mutant and transgenic lines has resulted in a gradually growing number of research areas taking zebrafish in use as model for human disease, environmental toxicology, high-throughput drug screening and more recently as model for aquaculture relevant research.

The maternal zygotic transition (MZT) in zebrafish coincides with the mid-blastula transition (MBT) and the zygotic genome activation (ZGA) after 10 transcription-independent cell cycles at 3 hours post fertilisation (hpf), manifested by a gradual loss of pluripotency and the on-set of differentiation. With the development of new sequencing technologies, transcriptomics and epigenomics it has been possible to uncover gene expression profiles, epigenetic landscapes and noncoding (nc)RNAs and their contribution to early development control. The transcriptome dynamics of over 8000 genes is characterized by different patterns of maternal mRNA degradation and activation of dormant deadenylated transcripts. Around 2000 cases of MZT isoform switches of transcripts expressed both before and after ZGA suggest a complex picture of early differentiation control. ncRNAs represent an increasing proportion of transcriptomes. The well described miR-430 family has an important role in maternal mRNA degradation control during the MZT. We are currently characterizing pre MBT embryo stages for the presence of other functional short ncRNAs. Methyl DNA immuno-precipitation (MeDIP)-chip and chromatin immuno-precipitation (ChIP)-chip analyses around MZT stage embryos revealed that promoter DNA methylation (5-methyldeoxycytdine; 5mdC) dynamics, in relation to changes in post-translational histone modifications and gene expression profiles, suggests a pre-patterning

of developmental genes consisting of a combination of DNA hypo-methylation and H3K4m3 on CG-rich promoters. During embryonic development the paternal genome is converted to 5-hydroxymethyldeoxycytidine (5hmdC), as part of DNA methylation reprogramming and 5hmdC also perhaps serves as an epigenetic mark. Using high performance liquid chromatography mass spectrometry to quantify global 5hmdC and 5mdC in zebrafish, low levels of 5mdC (1.9%) were found at 0.5 hpf which increased to 8% up to 96 hpf. No 5hmdC was detected before 12 hpf, but levels increased during development (0.23%). In summary there seem to be a complex interplay between several epigenetic signals during the preparation of the embryo for the MZT and the post MBT development.

Presenting author: Peter Aleström, peter.alestrom@nmbu.no

Liver organogenesis in zebrafish

M. Poulain, D. Stamataki, J Cayuso and E. A. Ober

Danish Stem Cell Center, University of Copenhagen, Denmark

A fundamental question in biology is how organs are formed during development. Specifically, how do organs with highly specialised cell types and distinct functions arise from a common pool of multipotent progenitors. For example, in the vertebrate digestive system the nascent foregut endoderm gives rise to the alimentary canal and its accessory organs, liver, pancreas, and the respiratory lungs. Despite the many essential physiological functions of the digestive organs, our knowledge of the mechanisms that control initial fate specification is limited. Still less understood are the morphogenetic processes governing the precise position of the organs within the digestive system, as well as their tissue architecture. Uncovering the principles underlying these processes is important for regenerative medicine, as they are likely recapitulated at least in part during organ regeneration and the differentiation of tissue-specific stem cells and the creation of artificial tissues. Our group combines zebrafish genetics with high resolution confocal imaging to investigate the gene regulatory networks that specify liver cells from the foregut and subsequently control their movements to assemble a liver bud and initiate organ outgrowth. We investigate the signals that control the cell fate choice between liver and pancreas focussing on Wnt and Bmp signals that originate from neighbouring tissues and are necessary for inducing hepatic fate. This raises the question as to how these signals interact to specify liver. We show that loss of both signals leads to a complete absence of liver, and conversely excess expression is sufficient to convert pancreas progenitors to differentiate into liver. Using a candidate approach, we identified a novel point of cross-talk between both pathways. Notably, we found that a known Bmp-inhibitor seems to facilitate Wnt signaling and liver specification in this context. In a second project we examine how the newly-specified liver progenitors move to form an organ bud in the right place and of the correct size. We identified two transmembrane proteins that control liver positioning in the body by coordinating the movement of the liver progenitors with those of the neighbouring tissues. Impaired gene function results in midline organs and malformed connecting ducts, phenotypes reminiscent of congenital defects in humans. Ongoing work of developmental regulators controlling liver progenitor specification and their subsequent movement mediating liver bud assembly and outgrowth will be presented.

Presenting author: Elke A. Ober, elke.ober@sund.ku.dk

Zebra fish as a model for inflammatory bowel disease (IBD): preliminary results

S. Haarder[1], P.W. Kania[1], P. Hyttel[2], T.L. Holm[3] and K. Buchmann[1]

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

[2]Anatomy Group, Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

[3]Department of Immunopharmacology, Novo Nordisk A/S, Måløv, Denmark

Preliminary results from our inflammatory bowel disease (IBD) model in zebra fish (*Danio rerio*) will be presented. Epidermal goblet cell depletion was seen in both oxazolone and TNBS-treated wild-type adult fish and to a lesser degree in control fish. This result is corresponding with findings in GI tracts of rodents with chemically-induced colitis. Using Optical Projection Tomography (OPT) we found an increased serotonin expression in oxazolone-treated intestines, suggesting a disturbance of signalling pathways. To further characterize the model, real-time polymerase chain reaction (qPCR) will be applied to study the expression of a multitude of IBD-relevant genes. Further, behaviour tracking of challenged and control fish will hopefully allow us to describe the model from a non-invasive point of view.

Presenting author: Simon Haarder, haarder@sund.ku.dk

Biosecurity in fish keeping facilities

S. E. LaPatra

Clear Springs Foods, Inc., Research Division, Buhl, Idaho USA

"Biosecurity" means a lot of different things to a lot of different people. Biosecurity is defined as implementing practices that minimize the risk of introducing an infectious disease and spreading it to the animals at a facility and the risk that diseased animals or infectious agents will leave a facility and spread to other sites and to other susceptible species. Biosecurity practices also reduce stress to animals, thus making them less susceptible to disease. Our policy emphasizes employee training, facility infrastructure, and animal and pathogen management. Well trained employees function as the first line of defense for any successful biosecurity program. There must be buy-in by the employees for any biosecurity program to be successful. Employees must be convinced on how this program is going to benefit the company, the animal and their job. Essential to the successful implementation of the biosecurity program are the adaptation of measures and techniques that are simple, effective and convenient. Excluding access of possible vectors onto farms is the next level of protection. Where possible, enclosures should be built over incoming water sources and farms with the objective of preventing "contamination" of the farm water supply and/or the farm itself. Good hygiene practices are essential for an effective biosecurity program for the prevention of the introduction and spread of infectious diseases. It is also important to understand and recognize the characteristics and signs of a disease and the pathogens that cause various diseases so steps can be taken to contain and solve the problem as soon as possible. The best way to prevent pathogen and disease dissemination on a farm is to prevent epizootics from occurring. Vaccinology and selective breeding should be emphasized along with passive fish handling and feed management. Probably the single biggest challenge is attempting to understand the environment that the fish are being cultured in. A better understanding of the culture environment and its variability will allow optimization of all the different strategies being implemented and aid in attaining the goal of your biosecurity program.

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

A search for new vaccine candidates in *Ichthyophthirius multifiliis*

L.vG. Jørgensen[1], P.W. Kania[1], K.J. Rasmussen[2], A.H. Mattsson[3] and K. Buchmann[1]

[1]Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
[2]Department of Cancer and Inflammation Research, University of Southern Denmark
[3]CBS, Technical University of Denmark

Considerable vaccine research efforts have been conducted with regard to the immobilization antigen Iag52b from the protozoan ciliate *Ichthyophthirius multifiliis* (Ich), which infects most species of freshwater fish. Fish that survive an infection acquire a subsequent level of immunity. The immunoglobulin IgM from immune rainbow trout and channel catfish targets the immobilization antigen on the surface of the parasite and during this process parasites become immobilized. Purified Iag52b injected together with Freund's complete adjuvant may induce up to 72% protection in vaccinated fish following challenge – however protection is only effective against one of the 5 parasite serotypes. Therefore, an immunogenic antigen, which protects against all 5 serotypes and which can be easily produced, is warranted. It has been shown that the mucosal immunoglobulin IgT from immune rainbow trout also binds to the parasite. In this context there is no evidence that Iag52b represents an important antigen. Therefore, experiments were conducted to elucidate which proteins IgT has affinity for. However, neither western blotting nor immunoprecipitation provided a clear result. In order to identify new antigens we have now turned to the use of the sequenced genome and the capabilities of neural networks. We have used a program that can predict potential vaccine candidates from the genome alone and select which ones may be suitable for production in a bacterial expression system. This has not been without challenges and 5 of the selected 14 proteins contained so many cysteines that we had to rethink the expression system. At the moment proteins are being produced in *E. coli* and in a baculovirus expression system. Focus as aforementioned has been on the antigen Iag52b and this protein came in as number 26 in the ranking of potential vaccine candidates analyzing the whole proteome of strain G5 comprising ~7200 proteins. We are at the moment testing the first 14 proteins on the ranking list.

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

Rainbow trout as a model in functional studies of anti-viral immunity

N. Lorenzen

Department of Animal Science, Aarhus University, Denmark

Salmonid rhabdoviruses like VHSV and IHNV are important pathogens in aquacultured rainbow trout due to their high infectivity and virulence. At the experimental level, this is an advantage in terms of the reproducible kinetics of infection trials following waterborne exposure to virus multiplied in fish cell cultures. This has allowed detailed *in vivo* studies of vaccine induced immunity as well as related protective mechanisms and concepts.

Vaccines based on plasmid DNA encoding the viral surface glycoprotein G under the control can induce long-lasting protective immunity when delivered by intramuscular injection in rainbow trout fingerlings. Vaccination of fish at an early stage appears advantageous, since larger fish require higher doses of vaccine to be protected. Even in fish with an average size of 0.5 g at the time of vaccination, good protection can be obtained. Interestingly, immunity is established already a few days after vaccination and cross-challenge experiments have revealed that protection in the early phase is non-specific. Later on, protection becomes very specific in terms of virus species. The protection in the early non-specific phase is related to interferon (IFN) induced defence mechanisms whereas specific antibodies and cellular components both play a role in the long lasting protection. The similarity of the functional immune response profile to that induced by a natural virus infection is striking and is most likely one of the major reasons for the efficacy of the rhabdovirus DNA vaccines. Although other elements like CpG motifs in the plasmid backbone sequence might play a role, the viral G protein appears to have an inherent ability to stimulate innate immune mechanisms by receptors and pathways that still remain to be characterized in detail. Expression of the rhabdovirus G protein on the surface of transfected muscle cells induces a histologically visible local inflammatory reaction with higher doses of VHSV G DNA vaccine. Cell surface expression may be important for a proper activation of the fish immune system, since blocking of the intracellular trafficking of the expressed glycoprotein G-gene interfere with protection. It was recently discovered that upregulation of certain micro RNAs is among the early innate response elements induced by VHSV infection as well as by DNA vaccination. Micro RNAs (miRs) interfere with protein expression at the posttranslational

stage by specific binding to the 3´UTR part of mRNAs. By injection of so called anti-miRs after innate immune stimulation followed by challenge with VHSV, it was possible to demonstrate that the highly upregulated miRs contribute to IFN induced protection. In the adaptive phase of protection, neutralizing antibodies are involved in protective immunity to VHSV in rainbow trout. Trout antibodies appear to neutralize the virus in a complement dependent manner, whereas mammalian IgG antibodies can neutralize the virus by themselves. Even small recombinant single chain antibody molecules can neutralize the virus *in vivo*. This was demonstrated in a gene-based delivery approach, which might have perspectives in human immunotherapy. Viral disease models in rainbow trout can thus be useful for conceptual studies both within and beyond the veterinary level.

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

The European sea bass as a model species in immunobiology

G. Scapigliati

Dept. DIBAF, University of Tuscia, 01100 Viterbo, Italy

The European sea bass (*Dicentrarchus labrax*), with a production of 150 kT (2012, from FAO), is the main non-salmonid marine species farmed in Europe. Due to its importance, the sea bass is a subject of intense research to investigate its physiology for evolutionary and applicative purposes. Of particular importance is the research on sea bass immunobiology, in order to protect the fish against microbial infections in farming conditions, and much information is now available on cellular and molecular immune defences, due to the availability of cellular and molecular markers. Regarding cell biology of leukocytes, there is a marked difference in the content of T cells and B cells among tissues, with T cells being preponderant in mucosal tissues and IgM-B cells in non mucosal tissues. The number of T cells is particularly high in the intestine, and can be modulated by changing the intestinal microbiome. The T cells are able to proliferate *in vitro* in response to either allo/xeno recognition and to lectins (conA, PHA), showing a tissue-dependent response. The B cells show antigen-specific serum IgM antibody responses, the presence of memory B cells can be elicited by systemic (intraperitoneal) administration of antigens, whereas mucosal administration of antigens (immersion) does not induce specific serum IgM antibody.

The deep-sequencing of transcriptomes from unstimulated thymus and gills provided useful information on expressed immune gene transcripts. The gills display an entire set of expressed genes coding for T cell populations (CD3, CD4, CD8) and subpopulations (Th1, Th2, Th17, Treg), as well as master regulators of T cell responses as they are known in mammals, and B cell populations (IgM, IgD, IgT). The intestine is a site of T cell expression and lymphocyte differentiation, where RAG-driven somatic recombination occurs in response to antigen administration. Attention is now focused on antiviral responses of sea bass against a main pathogen, the betanodavirus (VERv), and preliminary results show measurable variation in the transcription of antiviral genes in the gills in response to mucosal (immersion) vaccination. Overall, after two decades of studies, the sea bass is considered a marine fish model to investigate the immunobiology of fish and vertebrates.

Research funded by the EU (6FP IMAQUANIM, 7FP TARGETFISH).

Presenting author: Giuseppe Scapigliati, scapigg@unitus.it

Common carp as experimental animal model

M. Forlenza

Animal Science Group, Wageningen University, The Netherland

The common carp (*Cyprinus carpio*) is a freshwater fish and is one of the most cultured fish species in the world. Especially in China, common carp accounts for 2.5 million metric tons/year. It is not a high value fish but it's beautiful cousin, the koi carp, can reach values of 150.000 Euro per fish, making koi the most expensive fish in the world. In this presentation I will show you how studies on carp have helped to elucidate fundamental aspects of the fish immune system. Using various infectious pathogens and owing to the availability of several leukocyte-specific antibodies, we have gained insights into humoral and cellular responses of carp to pathogens. In our group, we have recently gained access to the sequenced carp genome, and thanks to the close phylogenetic distance of carp to zebrafish (*Danio rerio*) we can easily identify and annotate large numbers of immune genes. Finally, I will conclude by giving some examples on how we have been able to use our current knowledge for the development of experimental vaccines against carp viruses and how the combined use of carp and zebrafish animal models should be considered a perfect 'twinning' match for future research in fish.

Presenting author: Maria Forlenza, maria.forlenza@wur.nl

Atlantic salmon as an experimental model

B. Nowak

IMAS University of Tasmania, Australia

Atlantic salmon is a species closely related to rainbow trout which has been used as a model species for a long time resulting in well-developed research tools, some of them applicable to Atlantic salmon. Atlantic salmon has significantly higher commercial significance than rainbow trout. While it is native to the area of Northern Atlantic Ocean, this species is farmed on all continents with the exception of Asia and Antarctica. Atlantic salmon is the main fish species farmed in Australia. With its genome publicly released in June 2014 by the International Cooperation to Sequence the Atlantic Salmon Genome, Atlantic salmon is now even more attractive as a model species. This presentation will discuss the use of Atlantic salmon as a model species. Disease models developed for Atlantic salmon to study fish immune response, including yersiniosis and Amoebic Gill Disease and in vitro models using Atlantic salmon organs and cells will be also presented.

Presenting author: Barbara Nowak, b.nowak@utas.edu.au

Methods Used for the Evaluation of Vaccine Efficacy

S. E. LaPatra

Clear Springs Foods, Inc., Research Division, Buhl, Idaho USA

In order to evaluate vaccine efficacy accurately, functional measures such as adaptive, innate and/or mucosal immunity need to be evaluated along with the kinetics of protection and relative percent survival (RPS) using a robust and reproducible pathogen challenge system. The experimental design for the challenge should include replicates of each treatment group and mortality >60% in the mock vaccinated controls at the completion of the challenge. During the challenge the pathogen should be re-isolated from a portion of the mortality and quantitated to confirm the cause of death. Measures of vaccine efficacy that can be statistically analyzed include cumulative percent mortality (CPM), mean number of days to death (MDD) and RPS. The challenge methods that are commonly used include injection, immersion, co-habitation and oral delivery of the pathogen and each one has advantages and disadvantages. The co-habitation challenge system is probably the most "real life" but takes the most effort to develop a consistent and reproducible methodology. Serological methods for the evaluation of vaccine efficacy require determining the functionality of antibody or other serum component(s) that can be consistently and accurately measured. The "process" in determining the functionality of a serum component includes evaluating the susceptibility of survivors to re-infection using a controlled pathogen challenge system. If the survivors are resistant to re-infection then various serum components can be investigated and a measurement method developed. The functionality of this component can be verified using passive transfer and challenge studies. Once verified this factor could be used as an indicator of vaccine efficacy. Vaccine efficacy can also be assessed by evaluating the kinetics of protection using onset and specificity studies that call for challenging fish from different treatment groups at 100 and 400 degree days and also evaluating long duration protection. There is a need for the development of a standardized procedure for the evaluation of vaccine efficacy.

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

The parasitic copepod *Lernaeocera branchialis* negatively affects cardiorespiratory function in Atlantic cod (*Gadus morhua*)

J.W. Behrens[1], H. Seth[2], M. Axelsson[2] and K. Buchmann[3]

[1] National Institute of Aquatic Resources, Technical University of Denmark, Denmark

[2] Department of Biological and Environmental Sciences, University of Gothenburg, Sweden

[3] Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark

The female of the parasitic copepod *Lernaeocera branchialis* L. parasitizes the gills of Atlantic cod *Gadus morhua* and other closely related gadoids. Furthermore, the head and appendages penetrate into the ventral aorta and bulbus arteriosus of their host. The parasite's pathogenic properties primarily include penetration of the gill epithelia as well as feeding on blood and occlusion of blood vessels (Kabata 1979. Parasitic copepod of British fishes. Ray Society, London, UK). We here show that despite the potential for adaptive anatomical modifications to maintain cardiac output (Gamperl & Farrell, 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. J. Exp. Biol. 207: 2539-2550), *G. morhua* cardiorespiratory performance is severely affected following consecutive *L. branchialis* infections. This has the potential to negatively impact the postprandial metabolic response, reflecting the capacity for anabolic and catabolic processes in the post-absorptive state of fish.

Presenting author: Jane Beherens, jabeh@aqua.dtu.dk

Tuna as an experimental model

B. Nowak

IMAS University of Tasmania, Australia

Bluefin tunas (Atlantic Bluefin Tuna - ABT, Pacific Bluefin Tuna - PBT and Southern Bluefin Tuna - SBT) are highly evolved fish species, characterised by their large size, wide range, efficient swimming, thermoregulation and other physiological adaptations making them best adapted to life in open oceans and extensive oceanic migrations that they undertake during their lives.

These species have very high market value and some of them have been declared endangered or threatened. All of these species are ranched (cage grow-out of wild juveniles for a limited time) but only PBT life cycle was closed in 2002 by Kinki University Japan and this species is now produced by hatcheries for cage grow out. While it is not logistically possible to run in vivo experiments with SBT, this species is very useful for in vitro studies. This presentation will review research on SBT and PBT and their potential use as model species for a range of research areas, including aquatic toxicology.

Presenting author: Barbara Nowak, b.nowak@utas.edu.au

Barramundi and other Australian fish species as experimental models

B. Nowak

IMAS University of Tasmania, Australia

Barramundi, *Lates calcarifer*, is a fish species which is commercially important in a number of Asian countries and Australia. Barramundi grows very fast in a range of environmental conditions and has been also farmed in Europe and North America. This species is easy to maintain under laboratory conditions and it is amenable to experimental manipulation. Its compact genome (approximately 700 Mb) is one of the smallest genomes of farmed fish species. All this makes barramundi an interesting model species. Other species discussed in this presentation include sand flathead, *Platycephalus bassensis*, yellowtail kingfish, *Seriola lalandi*, and striped trumpeter, *Latris lineata*. In particular, the potential of these species to be used as models, in particular in aquatic toxicology and aquatic pathology, will be discussed.

Presenting author: Barbara Nowak, b.nowak@utas.edu.au

RNA interference (RNAi) as a possible control of whirling disease in rainbow trout *(Oncorhynchuss mykiss)*

S. Sarker and M. El-Matbouli

University of Veterinary Medicine, Vienna, Austria

Whirling disease caused by the myxosporean agent, *Myxobolus cerebralis*, is a problem in the European Aquaculture industry and poses a threat to the survival of wild rainbow trout *(Oncorhynchus mykiss)* in North America (El-Matbouli et al. 1999; Hedrick & El-Matbouli 2002; Hedrick et al 2003). The life cycle of *M. cerebralis* alternates between two transmission stages: an actinosporean triactinomyxon spore that develops in an aquatic oligochaete, *Tubifex tubifex* and *M. cerebralis* myxospores that develop in salmonid host (El-Matbouli et al. 1999). An experimental approach to control whirling disease by breaking down the life cycle of *M. cerebralis* in oligochaete host using RNA interference (RNAi) technology will be described. Specifically, how small interfering RNAs (siRNAs) will target important genes of *M. cerebralis* to: (i) hinder the development of the triactinomyxon actinospores in the oligochaete host or, (ii) to down regulate the expression of genes important for spore's pathogenesis in the oligochaete host to prevent it from infection of rainbow trout and subsequent development of whirling disease.

Presenting author: Subhodeep Sarker, subhodeep.sarker@vetmeduni.ac.at

Seabream as a model to elucidate evolution and diversity of the immune system

J. Galindo-Villegas and V. Mulero

The Fish Phagocyte & Cytokine Lab, Faculty of Biology, Murcia University, Spain

Fish represent the oldest and most diverse classes of vertebrates, comprising around the 48% of the known member species in the subphylum Vertebrata. The Teleost Gilthead seabream (*Sparus aurata*), which belongs to the wide spread order Perciformes, is a major farming species under intensive culture level in open waters of many Mediterranean countries. This condition turns it as a clear target for diverse opportunistic pathogens causing infectious diseases. However, until recently, the seabream immune defence mechanisms, and the ontogeny of their innate immune cells has been poorly understood. In this presentation I will comment on how we succeed, using this immunologically tractable fish model, to establish the correct timing in which innate and adaptive genes develop and achieve immunocompetence against specific antigens. To effectively discriminate among the diverse cell types involved in fish phagocytic defence, we produced and characterized specific antibodies (G7 and Mcsfr) against the two major professional phagocyte populations present in this species, namely acidophilic granulocytes (functionally equivalent of mammalian neutrophils) and monocytes/macrophages respectively. Furthermore, we gain insight into the phylogeny of the acidophilic granulocytes, and achieve to describe an outstanding evolutive process, the presence of biologically active histamine in mast cells which are recognized as important initiators and effectors of innate immunity. Thereafter, we explored for the presence and the spatial distribution of signaling pattern recognition receptors in leukocytes. Findings indicate that receptors are diversely located and display highly mammalian homology, despite the fish gene duplication phenomena. Additionally, using seabream macrophages we found "*ex-vivo*" a caspases-1 independent processing and release of the inflammatory marker IL-1β, and surprisingly, got to identify even a new member of the IL-1 family (IL-1Fm2). Thus, now our efforts are focused in developing a targeted mucosal vaccination strategy applying the immune knowledge in addition to strong adjuvants and designed toxoid preparations in order to increase seabream disease resistance against the widely spread disease pasteurellosis.

Presenting author: Jorge Galindo-Villegas, jorge-galindo@usa.net

European eel as experimental model I: Assisted reproduction technology and standardized fertilization methods for mass production of viable embryos and larvae

J. Tomkiewicz

DTU Aqua, Fish Biology Group, Technical University of Denmark, Denmark

Eels (*Anguilla* spp.) belong to the elopomorph superorder, a group of phylogenetically ancient teleosts that possesses very unusual features in their multi-stage catadromous life cycle as well as in their different ontogenetic stages. The one characteristic uniting this group of fishes is that they all have leptocephalus larvae, which are unique to the Elopomorpha. Furthermore, key aspects of their life cycle still rank among the great unsolved mysteries of biology, the most obvious being that there is still no direct observational evidence on the oceanic migrations of anguillid silver eels to their spawning areas and spawning itself. Even today, spawning sites are exclusively recognized by delimiting the occurrence of leptocephalus larvae on their journey towards growth areas in continental waters.

Prior to spawning migration, juvenile yellow eels enter the silvering process, where morphological and physiological changes take place; however, the migratory silver eels are still sexually immature. Sexual maturation and gonadal development in eels, as in other vertebrates, is regulated by gonadotropic follicle-stimulating hormone (FSH) and luteinizing hormone (LH) that are produced by the pituitary. However, in the silver eel stage, sexual development is suppressed by a dopaminergic inhibition at the brain-pituitary level. This is likely an adaptation related to their long migration to the spawning site, especially in temperate species, where e.g. the European eel (*A. anguilla*) migrate ~6000 km from the European continent to the Sargasso Sea. If prevented from undertaking their oceanic migration, sexual maturation and gonad development remains blocked by this dopaminergic inhibition of pituitary activity and absence of stimulation by Gonadotropin-Releasing Hormone. This inhibition must be released once the eels approach the spawning site, however the mechanism behind this physiological regulation remains unresolved.

The complex hormonal regulation of eel sexual maturation and gonadal development has motivated the choice of eel as an experimental model for studies on the development and physiological functions of the reproductive endocrine system. The knowledge generated through this research has given rise to methodologies applied in captive reproduction of eels, using salmon or carp pituitary extracts as sources of FSH and LH for induction of vitellogenesis in female eel and hCG for induction of spermatogenesis in male eel. In female eel, oocyte maturation that precedes ovulation is regulated by LH and the maturation-inducing steroid, 17α, 20β-dihydroxy-4-pregnen-3-one, DHP). Also this intricate process is induced hormonally by administration of e.g. DHP.

Challenges in European eel reproduction experiments include optimizing the efficacy of hormonal treatments, and thereby gamete production, quality and offspring developmental competence. In recent years, improvements in assisted reproduction technology including standardized fertilization methods has promoted mass production of viable eggs and larvae, which now allows experimental work on larval culture technology. Successful captive breeding and establishment of hatchery technology for commercial production of glass eels is fundamental to future sustainable aquaculture for this endangered species, and would allow the industry to rebuild the highly profitable market for eel aquaculture and suppliers.

Presenting author: Jonna Tomkiewicz, jt@aqua.dtu.dk

Improving biophysical rearing conditions during early life stages of European eel

S.R. Sørensen

Section for Population Ecology and Genetics, Technical University of Denmark, Denmark

Although the European eel are reared in aquaculture, this industry relies solely upon wild-caught juvenile glass eels that arrive to the European coasts after a 6000 km journey from the Sargasso Sea, where they were hatched. Within the European continent, eels are in an immature stage and although, attempted since 1930, utilizing maturational hormones, we only recently succeeded in refining reproduction protocols enabling rich quantities of viable gametes to support captive reproduction. In view of these obstacles, the last decade's research has shown substantial progress which builds on large effort to develop standardised protocols for fertilization which was not present. Milt and egg characteristics hold early clues on viability and we aimed at improving knowledge on characterizing gametes produced through induced maturation. We identified standardized sperm quantification tools and best ratios for mixing of sperm and egg prior to the activation of both gametes in the marine environment. Improper activation and swelling of *in vitro* produced eggs was frequently observed however and seen to negatively affect embryonic development and hatching. Consequently we aimed to establish improved biophysical conditions for the fertilization and incubation both establishing standardized experimental conditions and improving survival. We found activation salinities and salt types to influence both egg quality and egg diameters to species specific dimensions. Yet another aim was to lower high mortalities in late embryonic and early larval stages. Here we found microbial conditions being of pivotal importance by suppressing microbial coverage on eggs and microbial activity on both eggs, and larval and seeing a remarkably improvement in hatch and larval longevity which can be significantly improved. These important steps in the early life stages of European eel have been significantly improved through our work within the recent decade and render this species a potential new species being fully and sustainably produced in aquaculture.

Presenting author: Sune Riis Sørensen, srs@aqua.dtu.dk

Tilapia and poeciliid fishes as experimental models

M. Rubio-Godoy

Instituto de Ecología, A.C., Mexico

Tilapia (*Oreochromis* spp.) are African fish nowadays cultured worldwide. Poeciliid fishes ("guppies") are originally South American but have also been translocated globally with the aquarium trade. Both are interesting models for basic and applied parasitological research. In this talk, I will present examples of the sort of experimental data obtained with these fishes in Mexico – and elaborate on their implications. For instance, in farmed tilapia, the distribution and abundance of the monogenean *Gyrodactylus cichlidarum* seem to be regulated by host immunity. The study of gyrodactylids infecting poeciliid fishes in Mexico not only contributes to the inventory of a recognized biodiversity hotspot, but also provides clues to the phylogeographical co-evolution of hosts and parasites. Finally, digenean parasites (*Phyllodistomum* spp.) of Mexican poeciliids will be shown as a case study to advocate for the use of an integrative taxonomical approach; *i.e*., one that combines as many sources of information as possible to describe new species (morphological, molecular, microscopic, ecological, etc.).

Presenting author: Miguel Rubio-Godoy: miguel.rubio@inecol.mx

The non-structural proteins of piscine orthoreovirus, organizers of virus assembly?

H.M. Haatveit, I.B. Nyman, Ø.W. Finstad, M.K. Dahle and E. Rimstad

Faculty of Veterinary Medicine and Biosciences,
Norwegian University of Life Sciences, Norway

Piscine orthoreovirus (PRV) is associated with heart- and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon. Phylogenetic analysis of PRV has revealed a taxonomic placement within the family *Reoviridae*, most closely related to the genus *Orthoreovirus*. Reoviruses are spherical non-enveloped viruses with icosahedral capsids surrounding the double stranded RNA (dsRNA) genome. The PRV genome consists of 10 dsRNA segments distributed in the classical orthoreoviral groups of three large, three medium and four small segments. Mammalian orthoreovirus (MRV) has been studied extensively and is currently used as basis for constructing a model for PRV replication. The PRV segments M3 and S3 encode the non-structural (NS) proteins µNS and σNS. The NS proteins are believed to be involved in establishment of viral factories. Viral factories are intracellular compartments (inclusions) where replication, packaging and assembly of novel viral particles occur.

In this study, we have cloned the PRV NS proteins and tagged them in the N- and C-terminus for recognition by available antibodies. The tagged proteins were expressed in fish cell lines, and the subcellular localization and co-localization between the two NS proteins was studied by confocal microscopy. We also studied the ability of µNS to assemble and co-localize with two of the structural PRV proteins, λ1and µ2. Our results demonstrated that µNS forms dense globular inclusions much similar to viral factories when expressed in fish cells. µNS assembles and co-localize with σNS as well as the structural proteins λ1 and µ2. Future studies will include an attempt to characterize motifs necessary for inclusion formation in the µNS protein.

Presenting author: Hanne Haatveit, hanne.merethe.haatveit@nmbu.no

Effects of fish species composition on *Diphyllobothrium* spp. infections in subarctic brown trout and Arctic charr - is the three-spined stickleback a key species?

Kuhn, J. A., Knudsen, R., Kristoffersen, R., Amundsen, P.-A.

Department of Arctic and Marine Biology, University of Tromsø, Norway

Subarctic brown trout (*Salmo trutta*) populations are often heavily infected with plerocercoid larvae of the cestode genus *Diphyllobothrium*, assumedly as a consequence of piscivorous behavior. Three-spined sticklebacks (*Gasterosteus aculeatus*) are frequently preyed upon by trout and are commonly infected with *D. ditremum* and *D. dendriticum*. These parasites may transmit from fish prey to fish predators, and predation on sticklebacks may therefore be the main reason for the elevated parasite infections observed in piscivorous salmonids. As sticklebacks are easily caught prey, their presence might also enhance the abundance of piscivorous birds functioning as final hosts for the parasites. This would increase parasite transmissions and result in an overall higher parasite load in the lake. To explore possible effects of fish species composition and in particular the presence of sticklebacks on the diphyllobothrid infections of lacustrine salmonids, we contrasted data from eight brown trout populations representing four different fish species compositions (trout in allopatry, trout in sympatry with Arctic charr (*Salvelinus alpinus*), trout in sympatry with stickleback, and trout in sympatry with charr and stickleback). Our findings suggest that sticklebacks do in fact play an important role for the diphyllobothrid infections in lacustrine salmonid populations.

Presenting author: Jesper Kuhn, jesper.a.kuhn@uit.no

Challenge models to clarify effects of piscine orthoreovirus (PRV) infection in blood cells on salmon robustness for oxygen deprivation, smoltification, stress and secondary infections

M. Lund[1], O.W. Finstad[2], V. Aspehaug[3], S.M. Jørgensen[4], E. Rimstad[2] and M.K. Dahle[1]

[1]Norwegian Veterinary Institute, Oslo, Norway

[2]NMBU, Oslo, Norway

[3]Patogen AS, Ålesund, Norway

[4]NOFIMA, Ås, Norway

In the 1990s, heart and skeletal muscle inflammation (HSMI) were first reported in Atlantic salmon in Norway, and later in Scotland (Kongtorp RT. 2004, HSMI in Atlantic salmon. Ferguson HW. 2005). HSMI is characterized by epicarditis, myocarditis and myocitis in red skeletal muscle (Kongtorp RT, 2004. HSMI in Atlantic salmon). *Piscine orthoreovirus* (PRV) was found to be associated with the disease in 2010 by Palacios et al (PLoS One). Studies on PRV pathogenesis in Atlantic salmon performed by Finstad, Dahle, Rimstad and co-workers at NMBU and NVI demonstrated in 2013 that PRV massively infects red blood cells (RBC) prior to infection of the heart in HSMI. Links between PRV-infection and secondary infections, stress sensitivity and reduced smolt quality has been suspected from screening studies in aquaculture but not systematically tested. These connections could be partly be explained by the massive infection of RBC.

The collaborators of this current research project, HSMI-more, shall execute three controlled cohabitation challenge studies. The first challenge study aims to elucidate the effect of PRV infection on smoltification in Atlantic salmon. The second study will clarify the effect of PRV infection in RBC on sensitivity to oxygen deprivation and stress. The final challenge study will look at the interaction between PRV and salmonid alphavirus (SAV), the virus responsible for pancreas disease (PD), in a co-infection challenge model.

Presenting author: Morten Lund, Morten.Lund@vetinst.no

List of participants

First name	Surname	e-mail	Affiliation
Leonardo Mantilla	Aldana	leomantilla@uvigo.es	Edificio de Ciencias experimentales, Campus As Lagoas-Marcosende, Spain
Peter	Aleström	peter.alestrom@nmbu.no	Department of Basic Science and Aquatic Medicine, Norwegian University of Life Sciences, Norway
Azmi	Al-Jubury	azmi_mmbb@yahoo.com	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Manfrin	Amedeo	manfrin@izsvenezie.it	Fish Pathology Unit, Istituto Zooprofilattico Sper.le delle Venezie, Italy
Liu	Anjian		Branch Company Maoming, Tongwei Co., LTD., China
Qusay	Bahlool	qusay@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Simona	Bartkova	sibar@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Jane	Behrens	jabeh@aqua.dtu.dk	National Institute of Aquatic Resource, Technical University of Denmark
Morten Sichlau	Bruun	mosb@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Kurt	Buchmann	kub@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Chiara	Bulfon	chiara.bulfon@uniud.it	University of Udine, Department of Animal Science, Italy,
Erika	Burioli	erika.burioli@izsto.it	Fish Pathology Lab, Istituto Zooprofilattico Sperimentale del Piemonte, Italiy
Ejner	Børsting	ejner@borsting.dk	Bjæverskov, Denmark
Jiwan Kumar	Chettri	jkc@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Inger	Dalsgaard	inda@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Xiao	Dan		Animal Health Institute, Tongwei Co., LTD., China
Maria	Forlenza	maria.forlenza@wur.nl	Animal Science Group, Wageningen University, The Netherlands
Camilla	Frandsen	vhk351@alumni.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Jorge	Galindo-Villegas	jorge-galindo@usa.net	Department of Cell Biology and Histology, University of Murcia, Spain
Christina Hejl	Holm-Hansen	szh522@alumni.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Simon	Haarder	simon.haarder@gmail.com	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Hanne	Haatveit	hanne.merethe.haatveit@nmbu.no	NMBU veterinærhøgskolen
Dimitra	Iakovaki	mgb303@ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark

List of participants

First name	Surname	e-mail	Affiliation
Louise von Gersdorff	Jørgensen	lvgj@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Per	Kania	pwk@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
John Peter Hewa	Kinyage	johnkinyage@yahoo.com	National Food Institute, Technical University of Denmark, Denmark
Jesper Andreas	Kuhn	jesper.a.kuhn@uit.no	Department of Arctic and Marine Biology, University of Tromsø Norway
Rozalia	Korbut	gpt818@alumni.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Scott	LaPattra	scottl@clearsprings.com	Clear Springs Foods, Inc., Idaho, USA
Jens Lauritz	Larsen	ellen@jeanty.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Anne Hjørngaard	Lauritzen	ahl@biomar.dk	Biomar, Denmark
Thomas	Lidenstrøm	thi@ssi.dk	Statens Seruminstitut, Denmark
Niels	Lorenzen	Niels.Lorenzen@agrsci.dk	Department of Animal Science - Fish Health, Aarhus University, Denmark
Morten	Lund	Morten.Lund@vetinst.no	Section of Immunology, Norwegian Veterinary Institute, Norway
Lone	Madsen	loma@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Moonika Haahr	Marana	mmarana@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Sanaz	Mazaheri	shma@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Foojan	Mehrdana	foojan@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Rzgar Jaarfar	Mohammad	rezgarhadad@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Ole Sten	Møller	osmoller@gmail.com	Zoological Museum,
Kristian Ege	Nielsen	kristianege@gmail.com	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Barbara	Nowak	b.nowak@utas.edu.au	IMAS, University of Tasmania, Tasmania
Elke	Ober	elke.ober@sund.ku.dk	Danish Stem Cell Center, University of Copenhagen, Denmark
Niels Jørgen	Olesen	njol@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Maki	Otani	maki@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Egle	Petkeviciute	lvq519@alumni.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Marie	Plambech	mpla@aqua.dtu.dk	National Institute of Aquatic Resource, Technical University of Denmark

List of participants

First name	Surname	e-mail	Affiliation
Sebastian Nikitas	Politis	snpo@aqua.dtu.dk	National Institute of Aquatic Resources, Technical University of Denmark
Miguel	Rubio-Godoy	miguel.rubio@inecol.mx	Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México
Kasper	Rømer Villumsen	krv@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Subhodeep	Sarker	Subhodeep.Sarker@vetmeduni.ac.at	Clinical Division of Fish Medicine, University of Veterinary Medicine, Austria
Giuseppe	Scapigliati	scapigg@unitus.it	Department of Environmental Sciences University Of Tuscia, Italy
Jacob Günther	Schmidt	jacsc@food.dtu.dk	National Institute of Aquatic Resources, Technical University of Denmark
Priscilla	Seah	kpr134@alumni.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Jakob	Skov	jask@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Alf	Skovgaard	alf@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Jonas Steenholdt	Sørensen	xzd234@alumni.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Sune Riis	Sørensen	srs@aqua.dtu.dk	Section for Population Ecology and Genetics, Technical University of Denmark
Helena Elisabeth Hou	Thomassen	heht@vet.dtu.dk]	National Veterinary Institute, Technical University of Denmark
Helena Elisabeth Hou	Thomassen	heht@vet.dtu.dk	National Food Institute, Technical University of Denmark, Denmark
Liu	Tianqiang		Animal Health Institute, Tongwei Co., LTD., China
Jonna	Tomkiewicz	jt@aqua.dtu.dk	National Institute of Aquatic Resource, Technical University of Denmark
Niccolò	Vendramin	niven@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Donatella	Volpatti	donatella.volpatti@uniud.it	University of Udine, Department of Animal Science, Italy,
Albert	Willemsen	a.willemsen@noldus.nl	Noldus Information Technology bv, The Netherlands
Cheng	Xiaoru		Special Feed Research Institute, Tongwei CO., LTD., China
Guo	Yizhong		Tongwei CO., LTD., China
Cheng	Yuanfang		Livestock and Poultry Research Institute, Tongwei CO., LTD., China
Lara	Zheng		EU Project Innovation Centre
Bent	Aasted	bas@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark