

**Danish Fish Immunology Research Center**  
**DAFINET**  
**The Research School**  
**SCOFDA**

**Workshop**  
*Vaccination of early life cycle stages of fish*



**University of Copenhagen**  
**Faculty of Life Sciences**

**Auditorium 1-01**

**Bülowsvej 17**  
**DK-1870 Frederiksberg C**  
**Denmark**

**Danish Fish Immunology Research Center, DAFINET  
The Research School SCOFDA**

**Workshop: Vaccination of early life cycle stages of fish  
April 7 and 8, 2010**

**Programme and book of abstracts**

*Edited by Kurt Buchmann, Lars Holten  
Andersen and Per W. Kania, University of  
Copenhagen, Denmark*

*Printed by Frederiksberg Bogtrykkeri,  
Frederiksberg, Denmark*

**Danish Fish Immunology Research Center  
DAFINET  
The Research School  
SCOFDA**

**Workshop**

*Vaccination of early life cycle stages of fish*

**University of Copenhagen  
Faculty of Life Sciences**

**Auditorium 1-01**

**Bülowsvej 17  
DK-1870 Frederiksberg C  
Denmark**

# Program

## Wednesday April 7, 2010

12.00 Opening address by the DAFINET and SCOFDA leader Kurt Buchmann

12.15

Christopher Secombes

Scottish Fish Immunology Research Centre, University of Aberdeen,  
Aberdeen, Scotland

What have we learnt about the evolution of the cytokine network from studies in fish?

13.00 Lunch

13.45

Scott LaPatra

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

Brood stock immunization: Does maternal transfer of specific antibodies protect rainbow alevins?

14.30 Coffee break

15.00

Lone Madsen, Technical University of Denmark, Copenhagen, Denmark

*Flavobacterium psychrophilum* infections in rainbow trout: possible control methods

15.30

Oystein Evensen

Norwegian School for Veterinary Science, Oslo, Norway

The antiviral response of the salmonid alphavirus infected cell and viral counteractions

16.00

Barbara Nowak

University of Tasmania, Launceston, Tasmania, Australia

Parasitic diseases in Australian aquaculture

16.45 Discussion

18.00 Dinner

## Thursday April 8, 2010

10.45 Opening of the second day of the workshop by Kurt Buchmann

11.00

Chris Gould, Intervet/Schering Plough Animal Health, Aquaculture Centre, Essex, UK  
Development and use of an oral IPN vaccine in small fish

11.30

Barbara Nowak, University of Tasmania, Launceston, Tasmania, Australia  
Vaccination of Atlantic salmon against *Yersinia ruckeri*: Experiences from Tasmania.

12.00

Martin K. Raida, University of Copenhagen, Denmark  
Experiences from *Yersinia ruckeri* vaccination experiments of rainbow trout fry

Lunch 12.30

13.30

Lars Holten-Andersen, University of Copenhagen, Denmark  
Control of vaccination procedures: a serological approach

13.45

Alf Skovgaard, University of Copenhagen, Denmark  
Nematode infections of cod larvae: reasons and consequences

14.00

Csaba Szekely  
Veterinary Medical Institute, Budapest, Hungary  
Using early life cycle stages of bream for elucidation of myxozoan life cycles

14.15 Coffee break

14.45

Per W. Kania, University of Copenhagen, Denmark  
Tracking anthropogenic spread of pathogens by molecular methods

15.00

Rasmussen, K. J. U  
Department of Cancer and Inflammation Research  
Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark  
Characterization and comparison of immunological functions in the primary and secondary circulatory system in *Oncorhynchus mykiss*

15.15

Lorenzen, N

Technical University of Denmark, National Veterinary Institute, Århus, Denmark

Protective immunity to viral haemorrhagic septicaemia (VHS) can be induced in 0.5 g rainbow trout fry by DNA vaccination.

15.30

Tiina Korkea-aho

University of Stirling, Institute of Aquaculture, Stirling FK9 4LA, UK

The use of bacteria as immunostimulants in aquaculture

15.45 Discussion

16.00 Closing the DAFINET and SCOFDA workshop



# Participants

## **Aabo Akademi University, Biocity**

### **Laboratory of Aquatic Pathobiology, Åbo, Turku, Finland**

Eva Högfors-Rönholm

Krister Sundell

Tom Wiklund

## **AquaSearch, Denmark**

Torben Nielsen

## **Biomar A/S, Denmark**

Anne H Larsen

Niels Hjerimitslev

## **Clear Springs Foods Inc., Idaho, USA**

Scott E. LaPatra

## **Danish Aquaculture Organisation, Denmark**

Niels Henrik Henriksen

## **Department of Veterinary Disease Biology, University of Copenhagen, Denmark**

Alf Skovgaard

Jakob Skov

Kurt Buchmann

Lars Holten-Andersen

Louise von Gersdorff Jørgensen

Martin Kristian Raida

Per Walter Kania

Sanaz Mazaheri

Jiwan Kumar Chettri

Rezkar Jaafar Mohammad

Rasmus Demuth Heinecke

Qusay Zuhair Mohammad Bahlool

Moonika Marana Olsen

Malgorzata Bruzio

Jens Laurits Larsen

Bent Aasted

Jan Salomonsen

## **Department of Natural Sciences, University of Copenhagen, Denmark**

Jørgen Andreassen

Kasper Rømer Villumsen

**Faroe Marine Institute, Noatun, Faroe Islands**

Danjal Petur Højgaard

**Hungarian Academy of Sciences, Hungaria**

Csaba Szekely

**Institute of Aquaculture, University of Stirling, Scotland, UK**

Tiina Korkea-aho

**Intervet/Schering-Plough Animal Health, Denmark**

Jørgen Nylén

Chris Gould

**Istituto Zooprofilattico Sper.le delle Venezie, Fish Pathology Department, Adria  
Italy**

Amedeo Manfrin

**Mosul University, Iraq**

Zohair I F Rahemo

**National Veterinary Institute, Technical University of Denmark, Denmark**

Inger Dalsgaard

Lone Madsen

Kirsten Kaas

Lene Gertman

Niels Lorenzen

Ellen Lorenzen

Jesper Skou Rasmussen

Katja Einar-Jensen

Brian Dall Schyth

Sekar Larashati

Seyed Amir Hossein Jalali

**National Food Institute, Technical University of Denmark, Denmark**

Michael Engelbrecht

**Norwegian School of Veterinary Sciences, Oslo, Norway**

Oystein Evensen

**School of Life Sciences, Heriot-Watt University, Scotland (U.K.)**

Sharif M Sharifuzzaman

**St. Petersburg State Institute on Lake and River Fisheries (GosNIORKh)**

**St. Petersburg, Russia**

Maxim Doronin

**Tasmanian Aquaculture and Fisheries Institute, University of Tasmania**

Barbara F. Nowak

**University of Aberdeen, Scottish Fish Immunology Research Centre**

Chris Secombes

**University of Southern Denmark, Odense**

Karina Juhl Rasmussen

Karsten Skjødt

Charlotte Harken

**Non-institutional guests:**

**Læskovvej 235, 4632 Bjæverskov, Denmark**

Ejner Børsting

**Møllemarken 18<sup>1.th</sup>, 2880 Bagsværd, Denmark**

Katharina Kreissig

# ABSTRACTS

## DAFINET

**April 7 and 8, 2010**

### **Introduction and welcome address**

#### **Kurt Buchmann**

DAFINET and SCOFDA leader, University of Copenhagen, Faculty of Life Sciences, Frederiksberg C, Denmark

As leader of the Danish Fish Immunology Research Center ([www.dafinet.dk](http://www.dafinet.dk)) and the research school SCOFDA (Sustainable Control of Fish Diseases in Aquaculture) (<http://www.life.ku.dk/sitecore/content/Projects/SCOFDA.aspx>) at the University of Copenhagen I would like to express my sincere thanks to the audience for taking time to contribute to this two-day workshop on fish immunology with special emphasis on vaccination of early life cycle stages of fish. A special thank goes to the lecturers who will share their important results with us during the next two days.

The Danish Fish Immunology Research Center was established on January 1, 2009 through a grant from the Danish Research Agency (The Danish Research Council for Strategic Research) and comprises not only scientists at the University of Copenhagen but also researchers at the Technical University of Denmark, the University of Southern Denmark, The University of Aarhus, University of Stirling, Scotland, University of Aberdeen, Scotland, The Fisheries Research Service Marine Laboratory, Aberdeen,

Scotland, the Friedrich-Loeffler Institute, Insel-Riems, Germany and the Norwegian School of Veterinary Sciences, Oslo, Norway. Several private enterprises have joined the collaboration. Among these we are happy to welcome one of the leading feed manufacturers, Biomar Denmark, the pharmaceutical company Intervet/Schering-Plough which supplies a large part of vaccines used in aquaculture, the trout breeder and egg supplier Aquasearch Farm, The fisheries consultants at Fishlab and last but not the least the company Aquatic Diagnostics, Scotland. In order to keep the numerous scientists at the right track we have asked Dr. Scott LaPatra from the research laboratory at Clear Springs Foods Inc, Idaho, USA to give us a hand, good advices, instructions and criticism. Together with professor Tony Ellis at the University of Aberdeen Scott has taken their seats in the external advisory board. This comprehensive collaboration is likely to give fish immunologists and fish pathologists the tools for developing a new area of fish immunology based on molecular discoveries in the next four years.

We are running a number of research projects around a center focusing on the ontogenetic development of the fish immune system. This will lead to a better understanding of the early processes in the fish immune system and optimize vaccination technology even in very young fish.

I am sure that the founder of the first Veterinary School in Copenhagen, Peter Christian Abildgaard, who prepared the basis of this part of the University of Copenhagen in 1773 (KU-LIFE), would be happy to learn that fish again has gained a central seat in the sun at his beloved faculty. P. C. Abildgaard was a well trained fish parasitologists who did not only describe several famous fish parasites but was the first to describe a cestode life cycle involving fish. Fish topics have been included in the

curriculum not only at the first veterinary school (located in Christianshavn) but also later when the faculty moved to Frederiksberg in 1858 and since it merged with the old sister University of Copenhagen in 2007. The Faculty is now – together with four other Nordic universities in Norway, Sweden, Iceland and Finland conducting a M.Sc. education in Aquaculture ([www.nova-university.org](http://www.nova-university.org)) or ([http://www.nova-university.org/networks/aqua/aqua\\_1edu\\_2programme.htm](http://www.nova-university.org/networks/aqua/aqua_1edu_2programme.htm)). This collaboration provides the basis for fruitful interactions between bachelor students, master students, Ph.D. students and more advanced researchers. We hope that we will see many of these students in the research laboratories in the future.

The DAFINET/SCOFDA collaboration organizes two international workshops each year. At the April workshop 2010 we will hear about cytokine networks, maternal transfer of immunity, vaccination of fish, new vaccine formulations, disease problems in wild and aquacultured fish. I am sure that we will get nearer the answer of how old fish should be in order to respond to vaccination with a protective effect.

# **What have we learnt about the evolution of the cytokine network from studies in fish?**

**Chris Secombes**

Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen AB24 2TZ, UK. [c.secombes@abdn.ac.uk](mailto:c.secombes@abdn.ac.uk)

The repertoire of cytokines present in fish is becoming more apparent following the sequencing of the genome of a number of fish species. On the one hand, a complex cytokine network is clearly present, with many genes homologues of those known in higher vertebrates. On the other hand, some genes have less clear homology and may be the result of gene duplication events within fish groups, and some genes well known in higher vertebrates are not apparent. This suggests that many cytokine genes were present in early vertebrates prior to the fish–tetrapod divergence, and have expanded independently in different vertebrate groups since that time. This talk will review some of the key cytokine genes discovered in fish to date, and outline their sites of expression and function where known. Their potential application as markers of immune system function will also be considered.

# **Broodstock immunization: Does maternal transfer of specific antibodies protect rainbow trout alevins?**

**Scott E. LaPatra<sup>1</sup>, Ben R. LaFrentz<sup>2</sup> and Ken D. Cain<sup>3</sup>**

<sup>1</sup>Clear Springs Foods, Inc., Research Division

PO Box 712, Buhl, Idaho, 83316 USA

<sup>2</sup>United States Department of Agriculture, Agricultural Research Service,

Aquatic Animal Health Research Unit, Auburn, Alabama 36832 USA

<sup>3</sup>Department of Fish and Wildlife Resources, University of Idaho,

Moscow, Idaho 83844-1136 USA

Vaccination of young fish may be a possible means of reducing the occurrence of disease. However, mortality due to disease can occur at a life stage when vaccination is not possible either because the animal is not fully immunocompetent or an efficient delivery method of the vaccine is unavailable. Antibodies can be elicited and are detectable in adult rainbow trout (*Oncorhynchus mykiss*) against a variety of pathogens. Vaccination of broodstock could potentially enhance incorporation of specific antibodies into developing ova and may protect very young life stages of fish. One of the important functions of antibody in higher vertebrates is to provide immune protection to developing embryos. In mammals, immune antibody function is provided by the selective transfer of maternal antibody across the placental membrane. In birds, antibody is provided to the developing embryo within the yolk of the egg.

Two studies were initiated to determine 1) if immunization of adult rainbow trout broodstock stimulates transfer of specific maternal antibodies to fry through the egg, 2) if

these antibodies are detectable in laboratory assays, and 3) if an enhanced level of protection after pathogen challenge was observed in newly hatched fry obtained from vaccinated broodstock. Adult rainbow trout were tagged, bled, and immunized by intraperitoneal injection with a vaccine either against infectious hematopoietic necrosis virus (IHNV) or *Flavobacterium psychrophilum*, the etiological agent of bacterial coldwater disease (CWD) and rainbow trout fry syndrome (RTFS). Fish were booster vaccinated at 6 to 8 week intervals and monitored for the presence and titer of specific antibodies. Additional fish were mock immunized as controls. At spawning select crosses were made based on the antibody status of the adult fish. Fertilized eggs from each cross were incubated separately in specific-pathogen-free constant temperature spring water. Eggs and fry at different developmental stages were sampled for the presence of specific antibodies. Progeny from select crosses were challenged with a virulent strain of IHNV or *F. psychrophilum* at very early time points post-hatch. The results demonstrated elevated levels of antibody in the sera, eggs and sac-fry from vaccinated adults when compared to the mock immunized controls. Maternally transferred antibody appeared to decrease from the time of fertilization to baseline levels in very young post hatch sac-fry. Although specific antibody was shown to be maternally transferred to fry, this antibody did not result in protection from pathogen challenge. However, since there is evidence that some pathogens can be vertically transmitted, eliciting specific antibodies by vaccinating broodstock that enhances antibody transfer to eggs and sac-fry could reduce the potential of intra-ovum transfer of pathogens and manifestation of disease in very early and susceptible life stages of fish.

# ***Flavobacterium psychrophilum* infections in rainbow trout: possible control methods**

**Lone Madsen and Inger Dalsgaard**

Technical University of Denmark, National Veterinary Institute (DTU Veterinary),  
Division of Veterinary Diagnostics and Research, Frederiksberg, Denmark

High mortalities among rainbow trout (*Oncorhynchus mykiss*) fry are often the result of infections caused by *Flavobacterium psychrophilum*, and in Denmark this pathogen can be found in nearly all freshwater fish-farms. The infection is usually treated with antibiotics. At present, disease outbreaks can be controlled by the use of the antimicrobial agent florfenicol, whereas varying resistance patterns in *F. psychrophilum* to the licensed drugs oxolinic acid and trimethoprim/sulfadiazine are seen. The possibility of further changes in resistance patterns in *F. psychrophilum* to antibiotics demands the investigation of alternative treatment methods. Bacteriophage control of *F. psychrophilum* may constitute a realistic approach in the treatment of the infection. An experiment investigating the occurrence of bacteriophages and bacteria in rainbow trout during the initial stages of an infection showed that the phage and the bacterium were still found in some of the fish 10 days after infection, both in the group only injected with phages as well in the group injected with phages and bacteria. Other alternative treatment methods have been studied, among them increasing the water temperatures during the initial stages of a disease outbreak in fry.

A prophylactic measure like vaccination is also a possibility. An approved vaccine is not available yet, but several studies have been made with different *F.*

*psychrophilum* preparations in small scale trials. Currently good management and high hygienic measures on the trout farms are the fish farmers choice for avoiding disease outbreaks with *F. psychrophilum* or at least keep them low. Studies have shown that the establishment of bore-hole water recirculation systems for broodfish and fry as well as high hygienic measures including the disinfection of eyed eggs can minimise disease outbreaks.

## **The antiviral response of the salmonid alphavirus infected cell and viral counteractions**

**Øystein Evensen**

Norwegian School of Veterinary Science, Oslo, Norway.

Salmonid alphaviruses (SAV) are emerging viruses in salmonid aquaculture worldwide. Few studies have addressed the functional aspects of interferon (IFN)- $\alpha$ -induced antiviral responses in vivo or in vitro. In this study the IFN-alpha was cloned and expressed as recombinant protein and used for in vitro studies. SAV-3 infection in a permissive salmon macrophage cell line (TO cell) results in IFN-alpha and ISGs gene mRNA and protein upregulation. SAV-3 infection result in moderate macromolecular arrest in infected cells at early time post infection. Pretreatment (24h prior to infection) of TO cells with salmon IFN-alpha results in an induction of an antiviral state that inhibits the replication of SAV-3 and protects the cells against virus induced cytopathic effect (CPE), while post-infection treatment has minor effects. The

antiviral state coincides with a strong expression of Mx and ISG15 gene mRNA and Mx protein expression. When IFN-alpha is administered to TO cells 24h postinfection virus replication is not inhibited and cells are not protected against virus-induced CPE. We show that SAV-3 is sensitive to the preinfection antiviral state induced by IFN-alpha while SAV-3 successfully replicates in nontreated cells likely through diminishing the cellular responses induced by IFN-alpha.

## **Parasitic diseases in Australian mariculture**

**Barbara F. Nowak**

National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Launceston, Tasmania, Australia

Diversity of Australian mariculture reflects a wide range of environmental conditions and high biodiversity of the marine environment . The fish species farmed include Atlantic salmon, rainbow trout, Southern Bluefin Tuna, yellowtail kingfish, mulloway and barramundi. Striped trumpeter, groupers and cobia are farmed on a smaller, experimental scale. As many of these species are farmed in grow-out cages, they can be affected by parasitic diseases.

Amoebic gill disease (AGD) is a condition affecting some species of farm-reared marine fish caused by *Neoparamoeba perurans*. This disease has been reported from Australia, USA, Ireland, Spain, Scotland and New Zealand and in all these countries it has been associated with the presence of *Neoparamoeba perurans*. Amoebic gill disease (AGD) is the most serious health problem of farmed Atlantic salmon in Tasmania. However the

disease is also present in other hosts, one of the most recent records includes farmed ayu in Japan. Reservoir populations of the amoeba and the mechanism of transmission to farmed fish have not been elucidated. Preliminary investigation showed negative results for sediments and biofouling organisms in AGD affected area. However *N. perurans* DNA was detected in alcoholic washings of salmon lice *Lepeophtheirus salmonis* collected from salmon from an affected farm in the USA. Furthermore cross-infection with another species of sea lice *Caligus rogercresseyi* was reported during an AGD outbreak in Chile. This suggests that epidemiology of this disease may depend on the geographical locations.

Sea lice are one of the most significant health problems in mariculture worldwide. However, despite records of *Caligus* sp from salmonids farmed in Tasmania, these parasites do not cause any significant issues for farmed Atlantic salmon and rainbow trout in Tasmania, Australia . In contrast, outbreaks of mixed *Caligus* sp. (predominantly *Caligus chiastos*) infections were reported in ranched Southern bluefin tuna (SBT), Port Lincoln, South Australia with prevalence reaching 100% in experimental cages. The highest mean intensity of sea lice ever recorded on SBT was 495 (on 19 May 2008 in an experimental cage), which is more than ten-fold higher than the previously recorded maximum intensity of 41 (commercial cage). The number of sea lice on SBT has been associated with lower condition index and blindness as well as increased plasma cortisol and glucose. *Caligus chiastos* has also been reported on farmed mulloway (max prevalence 14.3%, intensity 1.5) and on farmed kingfish in South Australia. However, it does not currently cause any significant health issues in these species. *Caligus neunnonae* has been recently described from striped trumpeter farmed

in a land based research facility in Tasmania. *C. nuenonnae* was recorded at the research facility on two occasions at a prevalence of 22.3% (mean intensity 1.4) and 4.3% (mean intensity 1.0). This parasite was also recorded from striped trumpeter farmed in the sea cages with the maximum prevalence of 2.5% (mean intensity 1.0).

The chondracanthid copepod, *Chondracanthus goldsmidi* Tang, Andrews & Cobcroft, 2007, was recently identified as an ectoparasite on gills, inner opercula and in nasal cavities of cultured striped trumpeter, *Latris lineata* (Forster), and is the first member of this genus known to parasitise a cultured host. Adult *C. goldsmidi* was associated with extensive epithelial hyperplasia and necrosis. Pathological changes were most pronounced near the site where the parasite was attached at the time of sampling, with papilloma-like growths surrounding the entire parasite resulting in deformation of the filament. Mast cells were absent in healthy gills, in contrast numerous mast cells were identified in the papilloma-like growths. The number of mucous cells increased near the parasite attachment sites on both the opercula and gills. A significant up-regulation of three pro-inflammatory cytokines, TNF- $\alpha$ , IL-1 $\beta$  and IL-8 was found in the gills. Examination of head kidney cells revealed a significant up-regulation of TNF- $\alpha$ , but not IL-1 $\beta$  or IL-8. Conversely, the spleen cells showed significant up-regulation of both IL-1 $\beta$  and IL-8, but not TNF- $\alpha$ . These findings allow for more detailed investigation of the striped trumpeter immune response, in addition to providing insight into possible mitigation strategies for large scale infestation of *C. goldsmidi* on commercially cultured striped trumpeter.

# **Development and use of an oral IPN vaccine in small fish**

**Chris Gould**

Intervet/ Schering Plough Animal Health, Aquaculture Centre,

24-26 Gold Street, Saffron Walden, Essex, CB10 1EJ

United Kingdom [chris.gould@sp.intervet.com](mailto:chris.gould@sp.intervet.com)

In recent years severe clinical outbreaks of IPN in the early hatchery stages of salmon production have become an increasing issue in both the northern and southern hemisphere. In addition to the high levels of mortality which can occur, badly affected populations of surviving fish may also suffer impaired growth. This leads to increased cost of production, decreased smolt quality and poor seawater performance.

Whilst a number of different management techniques are routinely employed to reduce the likelihood of a clinical IPN infection in the hatchery, or the severity of an outbreak if it does occur, these have not proven to be completely effective in preventing clinical disease events. An effective vaccine which could be administered to these very small fish offers another tool to combat the disease.

Development of oral vaccines for fish has long been an important research focus for the global aquaculture industry. Clearly this route of administration offers many advantages in mass vaccination of fish over immersion or injection methods. This is even more true when attempting to vaccinate extremely small fish.

This paper will discuss the development of an effective oral vaccine delivery system for fish and use of this to develop a recombinant IPN vaccine, AquaVac<sup>®</sup> IPN

Oral, which is now being used to control IPN infections in commercial salmon hatcheries in Chile, Norway and the UK.

## **Vaccination of Atlantic salmon against *Yersinia ruckeri*:**

### **Experiences from Tasmania**

#### **B.F. Nowak**

National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Launceston, Tasmania, Australia

Bacterin-based enteric red mouth disease (ERM) vaccines and similarly prepared yersiniosis vaccines have been predominantly used to successfully control ERM in Northern Hemisphere rainbow trout and yersiniosis affecting Atlantic salmon cultured in the Southern Hemisphere. However, the emergence of new strains of the pathogen, *Yersinia ruckeri*, changing climatic conditions and increasing reports of vaccine failure highlight the need to re-evaluate the performance, design and use of existing *Y. ruckeri* bacterin-based vaccines. Yersiniosis killed half a million fish in just one hatchery during a 6 month period during 2007, resulting in a smolt shortfall for the entire Tasmanian salmon industry. In contrast to the *Yersinia ruckeri* isolates from Northern Hemisphere, Australian isolate serovar O1b is pathogenic to Atlantic salmon but not rainbow trout. While most Tasmanian Atlantic salmon are now (since 2006) vaccinated against yersiniosis (Yersinivac-B, developed by DPIW Tasmania, manufactured by Intervet), significant disease outbreaks still occur. Here, we propose to develop new vaccination strategy which will improve performance of the existing vaccine against yersiniosis. An

improved vaccine performance is crucial, not only because of an increased production cost due to yersiniosis (combined cost of mortalities, labour cost resulting from time to remove mortalities, treatment costs) but even more importantly because of fish welfare. We investigated effects of different of vaccine application methods. Survival after double dip, single dip, bath and ip vaccination was significantly better than control unvaccinated fish in a challenge 3 weeks post-vaccination. These results confirm that dip is better than bath although all vaccinated treatments have RPS values of >60%. The double dip vaccinated group has significantly better survival than the bath vaccinated group but there is no statistical difference between the single dip and bath. The IP vaccinated fish had a very high RPS of 95.5%. While IP vaccination maybe not practical for salmon industry, it provides a positive control for experimental research. The survival 12 weeks post vaccination further confirmed our previous results.

Preliminary measurements of specific anti-*Yersinia ruckeri* antibody levels, serum bactericidal activity and complement activity were assessed in the vaccinated and non-vaccinated fish. There was no apparent relationship between vaccination and serum antibody or between serum antibody and survival. However, there was a trend for the naive survivors to have greater body mucus lysozyme and gill mucus lysozyme concentrations, suggesting that at least for naive fish mucosal immune response maybe more important for survival than systemic response. This study is part of a larger project to improve vaccine performance through understanding molecular host-pathogen interactions in yersiniosis. Therefore, the conditions required for an effective yersiniosis vaccine as well as the future direction needed to investigate yersiniosis pathogenesis, host

immunity and the potential identification of genetic markers to predict vaccine efficacy are discussed.

## **Experiences from *Yersinia ruckeri* vaccination experiments of rainbow trout fry**

**Martin K. Raida**

University of Copenhagen, Faculty of Life Sciences, Department of Veterinary Disease Biology, Denmark

ERM caused by infections with *Yersinia ruckeri* are representing one of the most problematic diseases in freshwater farming of rainbow trout. All stages of trout including fry, juveniles and adults are susceptible to infection. Vaccination against this bacterial disease has been used through more than 30 years. Vaccination can be performed by intra-peritoneal administration, by the oral route and by immersion. At present an immersion vaccine, which can be used even for fry, confers reasonable protection under practical fish-farm conditions. The immune response of the host following immersion vaccination and challenge has been studied at different temperatures. The reactions comprise both innate and adaptive mechanisms. Temperature has a major impact on the reactions including protection.

# **Control of vaccination procedures: a serological approach**

**Lars Holten-Andersen**

University of Copenhagen and Technical University of Denmark, Denmark

There is a high demand for prophylactic vaccines inducing maximum protection against infectious diseases in farmed trout and salmon. The motivation factors are to limit the loss of fish and decrease the use of antibiotics. However, at Danish marine trout farms outbreaks of furunculosis, caused by *Aeromonas salmonicida*, are recurrent. This has raised the question whether the vaccines in use against furunculosis are good enough. Nonetheless, it is known from the literature that insufficient vaccination of a population with a highly effective vaccine is less protective than giving a vaccine with only 30-50% efficacy to the whole population (O'Callaghan et al, 1999). Hence, we decided to look at the vaccine protocols at the farms and not least the frequency of unvaccinated fish in the populations. For this purpose we developed a highly sensitive and specific assay (ELISA) for detection of antibodies against the vaccine antigens. It is the results from these investigations that will be presented.

O'Callaghan CJ et al, 1999. Predicting the effect of vaccination on the transmission dynamics of heartwater (*Cowdria ruminantium* infection). Preventive Veterinary Medicine, Vol. 42 (1), pages 17-38.

# Nematode infections of cod larvae: Reasons and consequences

**Alf Skovgaard<sup>1\*</sup>, Qusay Z. M. Bahloul<sup>1</sup>, Peter Munk<sup>2</sup>, Kurt Buchmann<sup>1</sup>**

<sup>1</sup> University of Copenhagen, Department of Veterinary Disease Biology, Laboratory of Aquatic Pathobiology, Stigbøjlen 7, DK-1870 Frederiksberg C. <sup>2</sup> Technical University of Denmark (DTU), National Institute of Aquatic Resources, Jægersborg Allé 1, DK-2920 Charlottenlund, Denmark.

The population of Atlantic cod in the North Sea has declined dramatically during the last 3-4 decades with one of the main causes being overexploitation through intense fishery. However, over-fishing may not be the only factor causing the decline of the cod. Recent climatic changes have led to elevated temperatures in the North Sea and these altered conditions are unfavourable to the North Sea cod. The exact mechanism behind the adverse effect of increased temperature on the cod population is under debate. A direct effect of temperature on the cod has been suggested, but it is also possible that the effect of temperature is indirect and that ecosystem modifications generated by climatic changes lead to reduced food resources for the cod. Particularly the possible indirect effects of climatic changes are unknown. Here we present evidence that prevalence of the parasitic nematode *Hysterothylacium aduncum* in larval North Sea cod was significantly higher in 2001 as compared with 1992 even though the cod stock had declined during this period. We anticipate that increased temperature has resulted in enhanced growth conditions for the parasite, thereby facilitating its dispersal and abundance. *H. aduncum* is presumably lethal to larval cod and thereby represents a climatically enforced

biological factor that may be important for the future size of the North Sea cod population.

## **Using early life cycle stages of bream for elucidating life cycles of myxosporean parasites**

**Székely C.<sup>1\*</sup>, Hallett S.L.<sup>2</sup>, Atkinson S.D.<sup>2</sup>, Molnár K.<sup>1</sup>**

Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest<sup>1</sup>

Center for Fish Disease Research, Oregon State University, Corvallis, Oregon, USA<sup>2</sup>

\*corresponding Author: e-mail [szekely@vmri.hu](mailto:szekely@vmri.hu)

The common bream *Abramis brama* is a good host for a number of parasites. The immune system in this fish host is relatively poorly described but it is known that fry and juvenile fish are especially susceptible to various pathogens. The small size and the susceptibility makes these fish stages valuable for parasitological research. The young stages are also suitable when performing life cycle studies on myxozoan parasites. They can easily obtain infection following exposure to the infective stage – the actinospore. This stage appears after an intraoligochaete development.

At least 6 *Myxobolus* species, distinguishable by morphology and site of development, infect the gills of common bream, *Abramis brama*. The life cycle of several of these species, such as *M. rotundus* were previously unknown. Therefore we initiated the present study in order to describe the life cycle of *Myxobolus rotundus*. When elucidating the myxozoan life cycles the access to uninfected bream fry is essential. For that purpose we collected wild sexually mature wild bream during the spawning season in Lake Balaton, Hungary. Eggs were stripped and fertilized already at the sampling sites. Fertilized eggs hatched and larvae were reared for 6 weeks

(fed only by *Artemia* nauplii and pelleted food) in experimental aquaria until they were used for the life cycle experiments. In the life cycle experiments, using the uninfected bream fry, the complete life cycle of *M. rotundus*, a gill parasite of bream, was elucidated. This myxosporean species also alternates with a triactinomyxon actinospore stage in the oligochaete *Tubifex tubifex*. We conducted successful *in vivo* infection trials and confirmed the identity of the alternate spore stages with DNA sequence data.

## **Tracking anthropogenic spread of pathogens by molecular methods**

**Kania P. W.<sup>1</sup>, Taraschewski, H.<sup>2</sup>, Han, Y.-S.<sup>3</sup>, Cone, D. K.<sup>4</sup>, Buchmann, K.<sup>1\*</sup>**

<sup>1</sup>Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark

<sup>2</sup>Zoologische Institut, Universität Karlsruhe, 76128, Karlsruhe, Germany

<sup>3</sup>Institute of Fishery Science, College of Life Science, National Taiwan University, Taipei, Taiwan 106

<sup>4</sup>Department of Biology, Saint Mary's University, Halifax, NS, Canada

The monogenean *Pseudodactylogyrus bini* parasitizes the gills of eels belonging to the genus *Anguilla*. Circumstantial evidence suggests that the parasite has been accidentally spread from the Pacific area (East Asia) to Europe by intercontinental eel trade. This is based on early descriptions of the parasites from Asian regions and the lack of records of the parasites in Europe before 1977. In addition, the susceptibility of European eels to

infections with the parasite is significantly higher compared to Japanese eels which could indicate that European eel had not undergone a co-evolution with this parasite. The present study was undertaken to elucidate the origin of the parasite by using molecular tools. Parasite samples were obtained from Europe (Germany), Asia (Taiwan) and Nova Scotia, the latter of which is the first record of *P. bini* in Canada. Sequencing of rDNA comprising part of the ITS 1 gene, 5.8S and part of ITS2 (1323bp) showed that *P. bini* isolates from the first two regions showed high variability. One sequence was found both in a number of Asian parasites and with one to a few transitions in European parasites which could indicate that they recently were split into the two regions. Other sequence variations suggested that one or a few genotypes of *P. bini* on one occasion were imported from Asia to Europe and that the two geographic isolates subsequently developed differently in the two regions. The Nova Scotian /Canadian isolates showed no variation and were found to be unique compared to the European and Taiwanese forms indicating that this population is independent in origin of the others. This could indicate that the Canadian parasites were introduced to North America on another occasion and independently of the European colonization.

**Characterization and comparison of immunological functions  
in the primary and secondary circulatory system in  
*Oncorhynchus mykiss***

**Rasmussen, K. J., Skjødt, K.**

Department of Cancer and Inflammation Research, Institute of Molecular Medicine  
University of Southern Denmark, Odense, Denmark

The secondary circulatory system (SCS) has mainly been described in ray-finned fishes (Actinopterygii) by Vogel (1985), Steffensen (1986) and Steffensen & Lomholt (1992). These authors suggested that the lymphatic system described so far lacked the characteristic anatomy normally seen in other vertebrates, especially mammals. Supported by dye-injected specimens, plastic casts and live images of the glass catfish (*Kryptoterus bicirrhis*), they showed that the SCS was in open communication with the systemic arteries via a large number of anastomoses of capillary dimensions. The SCS forms capillary beds situated in the outer surfaces of the fish such as the skin, gills, mouth and pharynx (Vogel (1985). On the other hand, the SCS appears to be absent in regions of the mesenteric and renal tissues, where the lymphatic system is normally found in mammals. Together with the presence of red blood cells (albeit in low numbers), these findings led to a reassessment of whether the SCC has true lymphatic function or not (Vogel (1985), Steffensen & Lomholt (1992), Skov and Bennett (2003)). An actual understanding of the SCS is still under debate, as the physiological function has not yet been completely elucidated. Furthermore it should be mentioned, that there is a

controversy between different research groups. Lawson et al. (2002) and Isogai et al. (2009) among others, thus argue that zebrafish does exhibit a lymphatic system similar to other vertebrates. In November 2009, we initiated a project aiming to characterize and compare the immunological functions in the primary and secondary circulatory systems of the rainbow trout (*Oncorhynchus mykiss*). The project will include identification of different cell types and -ratios in the primary circulatory system (PCS) vs. the SCS. To this end, we are currently in the process of generating specific antibodies against suitable cellular markers. Furthermore, the functional capacity of the classical- and alternative-complement pathways, and the mannose-binding lectin pathway will be determined in the SCS and compared with that of the PCS. Finally, a quantitative and qualitative comparison between sera from the primary and secondary circulation will be analyzed by means of proteomics.

Isogai S, Hitomi J, Yaniv K, Weinstein BM. (2009). Zebrafish as a new animal model to study lymphangiogenesis. *Anat Sci Int.* 2009 Mar. 14

Nathan D. Lawson and Brant M. Weinstein. (2002). In Vivo Imaging of Embryonic Vascular Development Using Transgenic Zebrafish. *Developmental Biology* 248. pp. 307-318

Skov PV, Bennett MB (2003) The secondary vascular system of Actinopterygii: interspecific variation in origins and investment. *Zoomorphology* 122:181–190

Steffensen, J.F. and Lomholt, J. P. (1992). The secondary vascular system. In: *The Cardiovascular System*, vol. X1IA (eds. Hoar, W. S., Randall, D.J., and Farrell, A. P.), pp.185-217. San Diego: Academic Press, Inc.

Steffensen, J. F., J. P., and Vogel, W. O. P. (1986). *In vivo* Observations on a Specialized Microvasculature, the Primary and Secondary Vessels in Fishes. *Acta Zoologica* 67, pp. 193-200.

Vogel, W.O.P. (1985). Sytemic vascular anastomoses, primary and secondary vessels in fish, and the phylogeny of lymphatics. *Alfred Benz. Symp.* Pp. 143-159.

## **Protective immunity to viral haemorrhagic septicaemia (VHS) can be induced in 0.5 g rainbow trout fry by DNA vaccination.**

**Lorenzen, N., Lorenzen, E. and Einer-Jensen, K.**

Technical University of Denmark, National Veterinary Institute, Århus, Denmark

Rainbow trout fry of an average weight of 0.5 g were vaccinated against viral haemorrhagic septicaemia (VHS) by intramuscular injection of 1 microgram of plasmid DNA encoding the VHS virus glycoprotein gene. Challenge with a lethal dose of virus at two different time points, 9 and 71 days post-vaccination respectively, revealed that a highly protective and lasting immunity was established shortly after vaccination, in accordance with earlier experiments with larger fish. The defence mechanisms activated by the DNA vaccine are thus functional at an early life-stage in rainbow trout.

## **The use of bacteria as immunostimulants in aquaculture**

**Tiina Korkea-aho**

University of Stirling, Institute of Aquaculture, Stirling FK9 4LA, UK

Beneficial bacteria, such as probiotics, can protect a host by competing for living space, adhesion sites and energy with pathogens by producing inhibitory compounds and by enhancing the immune response of the host. The majority of beneficial bacteria used for terrestrial animals are lactic acid bacteria, which acts beneficially for the gastrointestinal

microbial balance of the host. In aquaculture lactic acid bacteria are occasionally used, but instead a diverse range of organisms from the fish's own microflora has been evaluated. Furthermore, recent studies of probiotics in fish have shown that their main mode of action is stimulation of the immunological responses of the fish. Beneficial bacteria in aquaculture offer an attractive disease control method especially for early life stages of fish as they are administered as live supplements in feed or by immersion.

My research will focus on a range of potentially protective bacteria with effectiveness against *Flavobacterium psychrophilum*, which causes high mortalities especially for young stages of rainbow trout (*Oncorhynchus mykiss*). Some of the probiotics under investigation are known to affect the fish by enhancing immunity, but the effect on the development of immunity, especially adaptive immunity, in early stages are not well known. Furthermore, probiotics are reported to produce extracellular products (ECP) or outer membrane proteins (OMP), which work as bacteriocins against pathogenic bacteria. ECPs and OMPs have been studied from pathogenic bacteria and can provide useful information on vaccine development because they can induce the production of protective antibodies. However, comparison of the protective and pathogenic bacteria with regards to the production of ECPs and OMPs is rarely considered in fish probiotic research. My research is aimed at determining the mode of action of the protective bacteria as immunostimulants as there is the possibility that similar antigens may exist between beneficial (probiotic) and pathogenic bacteria, such as *Flavobacterium*, that may elicit a protective immune response in the fish against *Flavobacterium*.



**The KU-LIFE group at your service**