

**The Research School
SCOFDA**
*Sustainable Control of Fish Diseases in
Aquaculture*



Two day workshop on welfare and health of fish
Date: October 30 and 31, 2007

Time: 11.00-18.00

**University of Copenhagen
Faculty of Life Sciences
Auditorium 1-01
Bülowsvej 17
DK-1870 Frederiksberg C
Denmark**

Book of abstracts edited by Karl Pedersen and Kurt Buchmann

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Buchmann (kub@life.ku.dk)**

Foreword

The Research School SCOFDA was established at KU-LIFE in 2001 and has been performing Ph.D. courses and workshops 2-3 times a year since then. The main aim is to provide high quality education of researchers within aquaculture and health related aspects of fish production in the wild and in aquaculture systems. The advisory board and the supervisors of the research school comprise high quality scientists from Danish and international universities (DTU, KU, SDU, AU) involved in molecular biology, immunology, vaccinology, bacteriology, virology, parasitology, histopathology, physiology and environmental sciences. The SCOFDA workshop in October 2007 follows the path from previous years gathering participants from business, the academic world, post and undergraduate students and government officials. By conducting the two day workshop including discussions with scientific and social interactions it is the hope that we will contribute to future development of sustainable aquaculture both at the national and the international level.

Photos: The front cover: Inserted photo shows the problematic crustacean parasite *Argulus* which causes devastating infections in Danish put and take lakes used for recreational fisheries. This page: Niels Lorenzen vaccinating rainbow trout fry against VHSV.



Programme, SCOFDA Workshop
Diagnosis and control of fish diseases
KU/LIFE, October 30-31 2007

Tuesday, October 30

- | | |
|---------------|--------------------------------------------------------------------------------------------------------------------------|
| 11.00 – 11.15 | Welcome and opening of workshop by research school leader Kurt Buchmann |
| 11.15 – 12.00 | Invited guest lecture “Disease diagnosis in Australian mariculture” by Barbara Nowak |
| 12.00 – 13.30 | Lunch |
| 13.30 – 14.15 | Invited guest lecture: “Monitoring the welfare of farmed fish”, by Lars Helge Stien |
| 14.15 – 14.30 | Proteomics of epidermal mucus from <i>Salmo trutta</i> . Peter Højrup |
| 14.30 – 15.00 | Coffee break |
| 15.00 – 15.15 | Extracellular DNA in epidermal mucus from <i>Salmo trutta</i> . Knud Ladegaard Pedersen |
| 15.15-15.45 | Molecular systematics and ecology of anisakid nematodes. Simonetta Mattiucci |
| 15.45-16.00 | Fish sampling at Galathea 3 for basic immunological research. Niels Lorenzen |
| 16.00-16.15 | Identification of a protistan parasite of cod larva yolk sacs by rDNA sequencing. Alf Skovgaard |
| 16.15 – 16.45 | Protection of cultured <i>Cyprinus carpio</i> against a lethal viral disease by an attenuated virus vaccine. Mordi Haimi |
| 16.45-17.15 | Discussions and conclusion of first day’s programme |
| 18.00 – 22.00 | Dinner (Restaurant Kong Gulerod, Gl. Kongevej 142, Frederiksberg) |

Wednesday, October 31

- | | |
|-------------|---------------------------------------------------------------------------------------|
| 9.00 – 9.45 | Invited guest lecture: “Disease diagnosis in Australian mariculture” by Barbara Nowak |
|-------------|---------------------------------------------------------------------------------------|

9.45 – 10.00	New Community legislation in aquaculture, how should Council Directive 2006/88/EC be implemented in real life. Niels Jørgen Olesen
10.00 – 10.30	Coffee break
10.30 – 11.00	Parasites of wild and farmed cod in Norway: the CODPAR project. Ken MacKenzie
11.00-11.15	Rainbow trout farms with a high degree of recirculation: pathogenic bacteria and antimicrobial resistance. Morten Sichlau Bruun
11.15-11.30	Skin lesions in Danish rainbow trout farms. Torsten Snogdal Boutrup
11.30-11.45	Bacterial pathogens in Danish rainbow trout mariculture. Karl Pedersen
11.45-12.00	Bath vaccination of rainbow trout against <i>Yersinia ruckeri</i> : effects of temperature on protection and gene expression. Martin Raida
12.00 – 13.30	Lunch
13.30 – 14.15	Invited guest lecture: Welfare of farmed fish from harvest to killing - meeting the future challenges” by Lars Helge Stien
14.15 – 15.15	Coffee break
15.15 – 15.30	Field-testing of a DNA vaccine against VHS in rainbow trout: Final results and perspectives. Ellen Lorenzen
15.30-15.45	Temperature-dependant expression of immune-relevant genes in rainbow trout following <i>Yersinia ruckeri</i> vaccination. Martin Raida
15.45-16.00	Seasonal dynamics of Seasonal dynamics of <i>Dactylogyrus</i> spp. (Monogenoidea) from the gills of Pool barb, <i>Puntius sophore</i> in the river Gomti, Luknow (India). Amit Tripathi
16.00 – 17.00	Discussions and conclusion of workshop

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ABSTRACTS

DISEASE DIAGNOSIS IN AUSTRALIAN MARICULTURE

Barbara F. Nowak

*School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania and Aquafin
Launceston, Tasmania, Australia*

Australia extends through a range of climatic zones and has a diverse mariculture industry. Recent fast development of cage mariculture resulted in outbreaks of diseases, many of them parasitic, some of them undescribed. Atlantic Salmon Gill Disease (AGD) is the most significant health problem affecting Atlantic salmon culture in Tasmania. Recently we have identified *Neoparamoeba perurans* as the causative agent of Amoebic Gill Disease. Yellowtail Kingfish industry is significantly affected by monogenean skin fluke *Benedenia seriolae*. Current treatment for this parasite is hydrogen peroxide. Marine hatcheries, in particular snapper and barramundi have occasional *Amyloodinium* sp. outbreaks. In sea cages, both snapper and barramundi can be affected by monogeneans, including *Neobenedenia melleni*, *Benedenia* spp. and *Anoplodiscus cirrusspiralis*.

Southern Bluefin Tuna are caught wild and fattened in sea cages for 3-6 months. While a number of parasites can be detected during the grow-out, only *Uronema nigricans* and *Caligus* sp. 1 have been associated with any losses. Normally free-living ciliate, *Uronema nigricans* infects fish through olfactory rosette and migrates to the brain. It causes swimmer syndrome, during which the fish come to the surface, change their colour to light brown and swim vigorously around the cage, followed by short bursts of forward movements with their heads out of the water until death. *Caligus* sp. 1 infections have been associated with eye damage and blindness. Other parasites on farmed tuna included blood fluke *Cardicola forsteri* and gill parasites: monogenean *Hexostoma thynni* and two species of copepods: *Pseudocycnus appendiculatus* and *Euryphorus brachypterus*.

Experimental culture of striped trumpeter, *Lateolabrax lineatus*, has been affected by myxozoan *Myxobolus neurophila* and two copepods: a chondracanthid and *Caligus* sp. 2. These parasites have been described recently. Currently, control and treatment methods are being investigated.

In Australia, most diseases are identified using a range of diagnostic methods, usually including histology and microbiology or parasitology. Disease diagnosis can be difficult without accurate identification of the causative agent. Diagnostic criteria and case definitions must be developed for each disease. This presentation focuses on diagnosis of disease in Australian mariculture, including identification of parasites.

MONITORING THE WELFARE OF FARMED FISH

**Lars Helge Stien, Ole Folkedal, Trygve Gytte, Tore Kristiansen, Jonathan Nilsson,
Thomas Torgersen**

The Animal welfare group, the Institute of Marine Research (IMR), Norway

Farmed fish are now being recognized as beings with more than a “three-second-memory”. This is supported by recent studies by the Animal welfare group at the Institute of Marine Research (IMR), which have shown that Atlantic salmon, Atlantic cod and Atlantic halibut are all able to be delay and trace conditioned. This indicates that farmed fish have some level of sentience and should be treated accordingly. There is also an increasing focus on fish welfare from consumers, animal welfare organisations and retailers.

Fish respond to stress with changes in behaviour. One example of this is that fish exposed to a sudden fright stressor respond with flight reactions and various changes in swimming and schooling behaviour. Behavioural responses are often immediate and have therefore the potential to provide early warnings of deteriorating conditions. One of the goals of the Animal welfare group is therefore to identify reliable behavioural indicators that can be used to monitor and quantify acute and chronic stress levels in farmed fish. An important part of this is the development of an internet based system for systematic recording of fish behaviour and other related environmental conditions in the sea cage, feeding practices, disease status, husbandry and more. The data from this system is stored in a central database at IMR. The final goal is to develop expert software that based on the data included in the database gives a good assessment of current and future fish welfare in the respective sea cage and also instructs the farmer in which actions should be made to improve the welfare of the fish if necessary.

An addition to this work is the development of an automatic system for continuous profiling of environmental conditions in sea cages from surface to bottom. The system comprises of a probe that is regularly winched down in the water column. The probe sends measurement data to a GSM terminal by radio every time it reaches the surface. This terminal also controls the winch. On receiving data the GSM terminal sends it onwards as a message to the database at IMR, where the data is analysed by the expert software. The measurement data and the result of the analysis are then transmitted to the person in charge of the farm via an internet webpage so that they can take appropriate action.

PROTEOMICS OF EPIDERMAL MUCUS FROM *SALMO TRUTTA*

Peter Højrup

Peter Højrup; Institute of Biochemistry and Molecular Biology, University of Southern Denmark

FISH SAMPLING AT GALATHEA 3 FOR BASIC IMMUNOLOGICAL RESEARCH

Niels Lorenzen*, **Martin K. Raida***, **Katja Einer-Jensen***, **Thomas Lindenstrøm*****, **Ellen Lorenzen***, **Kurt Buchmann****, **Brian Dall Schyth***, **Per W. Kania****, and **Niels Jørgen Olesen***.

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****Statens Seruminstitut, Copenhagen.*

As a part of the Danish Galathea 3 research expedition 2006-2007 we participated with a project entitled "Search for the origin of the vertebrate immune system". Based on sampling of tissues related to the fish immune system and subsequent phylogenetic analysis of selected key immune elements, this project aims at chasing and characterising the evolutionary origin of the immune defence mechanisms against infectious diseases in higher vertebrates. We hereby aim to create new knowledge of how the vertebrate immune system has developed. Fish comprise the greatest group of vertebrate species and they have adapted to many different habitats such as the Arctic, the tropics and the deep sea. In the same time, fish represent the earliest class of vertebrates possessing the molecular key elements and functions of an adaptive immune system as known in higher vertebrates such as mammals, including man. By identification and characterisation of key molecules of the fish immune system in a variety of both primitive and advanced fish species adapted to different life conditions, we will display the molecular spectrum of the immune system in this early class of vertebrates and hereby discover how the basic elements of the immune system known in mammals today have evolved. Since hosts and pathogens have developed hand in hand, we will also analyse the pathogens associated with the fish. The project will create a scientific background for a better understanding of how the immune system is able to defend vertebrates against infections, and hereby provide valuable information for improvement of disease prophylaxis in cultured fish, terrestrial husbandry animals and man. During our participation of the expedition, starting from Sydney, Australia, and via Solomon Islands to New Zealand, across the Pacific to the Antarctic Peninsula, up north to the Caribbean's via Panama and ending in Boston, USA, a total of 305 tissue samples were collected, covering 79 different fish species in 24 different taxonomic families. On top of this a number of samples were collected for virological and parasitological examination. Illustrations from the cruise across the Pacific, from New Zealand to Chile will be presented.

EXTRACELLULAR DNA IN THE EPIDERMAL MUCUS FROM *SALMO TRUTTA*

Knud Ladegaard Pedersen

Institute of Biology, University of Southern Denmark:

MOLECULAR SYSTEMATICS AND ECOLOGY OF ANISAKID NEMATODES

Simonetta Mattiucci

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Research on sibling species of nematode parasites has increased exponentially over the past two decades, for a large part by the increasing availability of the application of genetic/molecular markers. Most nematode descriptions conformed with that which can be regarded as the morphological or typological species concept. Speciation is not always accompanied by morphological change, the true number of biological species is likely greater than the current tally of nominal species, most of which are delineated on purely morphological grounds. The detection of sibling species has challenged parasitologists in terms of an expected genetic variation and heterogeneity within a nominal species, and has led to the definition of a parasite species in accordance with Mayr's "biological species concept" (BSC) (Mayr *et al.*, 1970). Using the BSC, sibling species are considered to exist when they are reproductively isolated and gene flow between them is interrupted. This species concept (BSC) was well tested by the application of allozyme markers in several anisakid nematodes. Indeed, during the last two decades, the number of species belonging to the genera *Anisakis*, *Pseudoterranova* and *Contracaecum* has increased due to the detection of several sibling species within various nominal species of these genera, i.e. taxa previously considered cosmopolitan and euryecious. Reproductive isolation and the absence of gene flow were demonstrated by the application of genetic markers (allozymes) between sympatric and allopatric sibling species, validating their specific status. The existence of sibling species of anisakid nematodes was later confirmed by the application of other DNA markers. Here the species of anisakid nematodes belonging to those genera detected by combining data obtained from two different genetic/molecular approaches: allozymes (20-24 loci) and DNA sequences and the mitochondrial gene of cytochrome oxidase-2 (mtDNA *cox-2*) are summarized. Estimates of their divergence at the nuclear and mitochondrial level obtained from different distance indices are reviewed. Ecological evidences, relating to the distributional range of the detected sibling species and their host preferences, and evolutionary aspects represent data-sets, that can be used for species delimitation and definition, are also presented.

IDENTIFICATION OF A PROTISTAN PARASITE OF COD LARVA YOLK-SACS BY rDNA SEQUENCING

Alf Skovgaard

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We have isolated material and obtained ssu rDNA sequences from a known, but hitherto unclassified, protistan of cod (*Gadus morhua*) eggs and larvae. Although first reported in 1992, it has so far not been possible to identify this parasite due to its limited and non-specific morphological and ultrastructural characters. Ssu rDNA unambiguously identifies the parasite as *Ichthyodinium*, a genus of dinoflagellate-like parasites that is known to infect a dozen of warm water, marine fish species worldwide. The cod parasite appears genetically identical to *Ichthyodinium chabelardi* which infects eggs and larvae of the Atlantic sardine (*Sardina pilchardus*) and bogue (*Boops boops*) in Portuguese and Mediterranean coastal waters. The ssu rDNA gene of *Ichthyodinium chabelardi* is, in turn, 97% identical to Asian isolates of a similar parasite from eggs of yellowfin tuna (*Thunnus albacares*) and leopard coral (*Plectropomus leopardus*). *Ichthyodinium* spp. are undoubtedly common and often overlooked parasites of fish eggs and larvae, and high mortalities of eggs due to this parasite has been observed both in its natural environment and in aquaculture facilities. Having identified a unique gene sequence of *Ichthyodinium* it is now possible to use molecular techniques for detecting and studying life cycle of the parasite.

PROTECTION OF CULTURED *CYPRINUS CARPIO* AGAINST A LETHAL VIRAL DISEASE BY AN ATTENUATED VIRUS VACCINE

*Mordi Haimi - Ms.c.
MAGNOY Ltd. Israel*

Massive mortality of Koi and common carp- *Cyprinus carpio* – has been observed since 1998 in many countries worldwide, resulting in severe economic losses. The cause of the disease is a large DNA virus KHV belonging to the herpes virus family. Prof. **Moshe Kotler** from the department of Pathology, The Hebrew University-Hadassah Medical School, Jerusalem and his team, demonstrated that the wild type KHV lost its pathogenicity following serial transfer in cell culture, and the clones isolated from the attenuated population could be used as prophylactic vaccine. In this presentation I will describe how the outbreak of KHV in Israel in 1998 affected the common carp and Koi carp industry. Further, it will be mentioned what the Israeli aquaculture industry learned from the spread of this infection. The talk will describe the measures taken by the industry. Special focus will be given to the attenuated virus vaccine, how the aquaculture enterprises in Israel are using this vaccine and finally elucidate the safety procedures in producing the vaccine.

DISEASE CONTROL IN AUSTRALIAN MARICULTURE

Barbara F. Nowak

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Mariculture in Australia covers a wide range of fish species. Southern bluefin tuna and Atlantic salmon are the two species cultured in seawater, there are also commercial companies growing barramundi, snapper, yellowtail kingfish and research into new species for aquaculture which includes striped trumpeter, dhufish and mullet. This large number of species provides an interesting challenge for fish health researchers as well as manufacturers.

Infectious disease can cause significant problems in fish culture. Immunomodulation, enhancing immune response by diet and use of immunostimulants, vaccines and adjuvants, is often required to continue sustainable (environmentally and economically) fish production. Many viral and bacterial diseases which are the main problems for aquaculture in Northern Hemisphere are absent from Australia. Only a few vaccines, mostly bacterial, are currently used. Traditionally they were all applied as immersion vaccination, but recently injection vaccination is becoming more popular. This presentation will summarise main ways to improve immune response in culture in Australia, including vaccination, use of immunostimulants and adjuvants. The examples used will be based on recent research at the University of Tasmania, including work on vaccines and adjuvants for Atlantic salmon, *Salmo salar*, vaccines and adjuvants and vaccines for barramundi, *Lates calcarifer* and immunostimulants for *Pagrus auratus*. Potential for immunomodulation to control amoebic gill disease in salmonids will be discussed. Improved knowledge and understanding of parasitic conditions will result in better control and treatment and thus increasing profitability and sustainability of mariculture.

NEW COMMUNITY LEGISLATION IN AQUACULTURE, HOW SHOULD COUNCIL DIRECTIVE 2006/88/EC BE IMPLEMENTED IN REAL LIFE

Niels Jørgen Olesen

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PARASITES OF WILD AND FARMED COD IN NORWAY: THE CODPAR PROJECT

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Cod farming is a rapidly developing industry in Norway. The standing stock of cod in aquaculture has increased markedly in recent years, from about 2000 metric tonnes in 2002 to about 13,000 in 2005 – a steep increase compared to other aquacultured species. A major concern is the possible effects of exchange of parasites between farmed and wild cod stocks. The aim of the CODPAR study is to develop a reference database on parasites of farmed and wild cod in the early stages of cod farming in Norway. The screening involves a complete parasitological examination of all organs of freshly killed farmed and wild cod from the same location, using a standard protocol. Farmed cod include both hatchery-reared and wild-caught cod ongrown in farms. Samples are taken from several widely dispersed sampling locations, and a spring and an autumn sample is screened from each site. In total, 244 cod from one site in North Norway and three from Western Norway have been examined. To date, a total of 45 parasite taxa have been recorded but, because many of the parasites could not be identified to the generic or higher taxonomic level, the actual number of species present must be at least 50. At least two are new species, while three others are new host records for cod. Wild cod were infected with the largest number of different parasite taxa (39) and hatchery-reared cod with the lowest number (19). Parasites transmitted via sea lice are significantly less common on hatchery-reared cod. The most significant results so far are: (1) occurrence of *Caligus* spp. (sea-lice) on farmed cod; (2) the significantly larger numbers of monogenean *Gyrodactylus* spp. on the gills of farmed cod; (3) the first record of the pathogenic protozoan *Ichthyobodo necator* on farmed cod; and (4) the high level of infection with the protozoan *Zschokkella hildae* in the kidneys and bladders of farmed cod. Parasites likely to cause problems in cod farming in the future are highlighted.

RAINBOW TROUT FARMS WITH A HIGH DEGREE OF RECIRCULATION: PATHOGENIC BACTERIA AND ANTIMICROBIAL RESISTANCE

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Fish farming in Denmark is going through changes to manage an increased production of rainbow trout (*Oncorhynchus mykiss*) and simultaneously reducing environmental impact. Eight traditional flow-through farms have been redesigned to “model farms” based on recirculation technology. The farms only take in well-filtered water and the amount of water used is very low due to a high degree of recirculation. One part of the project is a Veterinary Practice concerning optimal veterinary routines and handling of diseased fish – including preventive measures, and monitoring of fish pathogens at the farms. Bacterial diseases giving rise to problems at trout farms in Denmark are caused by *Flavobacterium psychrophilum* and *Yersinia ruckeri*, *Aeromonas salmonicida* and other pathogens are occasionally isolated. Bacteriological examinations of 20 different sizes) were done on each model farm quarterly. Samples were taken from skin, gills, spleen, kidney and pathological changes if present, and inoculated on 2 different agar plates, blood agar (for the isolation of *Y. ruckeri* and *A. salmonicida*) and tryptone yeast extract salts (TYES) agar for the isolation of *F. psychrophilum*. Samples from skin and gills were also inoculated on TYES agar added sulfadiazin and trimethoprim, thus suppressing the natural water flora but supporting growth of *F. psychrophilum*. It was possible to isolate bacterial species (sometimes all three species in the same fish), both from fish with and without disease symptoms. *F. psychrophilum* was isolated from all eight farms, *Y. ruckeri* from seven of the eight farms, whereas *A. salmonicida* was only isolated from three farms. The antimicrobial susceptibility/resistance pattern of the isolated bacteria is presented, and the importance of the isolated bacteria is discussed. So far, our results show that pathogenic bacteria can be found at the “model farms”; however, it seems that disease outbreaks caused by bacterial infection are reduced in comparison to traditional fish farms with earth ponds.

SKIN LESIONS IN DANISH RAINBOW TROUT FARMS

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Changes in the skin of rainbow trout can be associated with ectoparasites, e.g. *Ichtyobodo necator* and *Ichthyophthirius multifiliis*, fungi, e.g. *Saprolegnia* spp., bacteria, e.g. *Flavobacterium* spp. and *Aeromonas* spp., or environmental factors, e.g. water oxygen level and pH of the water. While ectoparasites and environmental factors often give rise to changes like pale discolouration, excess mucus production and mild epidermal hyperplasia, bacterial and fungal infections often give rise to more severe lesions with ulceration, necrosis and pronounced inflammatory reaction. During the summer of 2007 we have experienced a number of cases with skin lesions among rainbow trout of 100-500 g in earth ponds and channels. Fish were most often seen with mild to moderate scale loss and thickening of the epidermis. Affected areas were yellowish and with pin-point haemorrhages, in a few fish more severe lesions were seen. An interesting finding was that the thickening seemed to be caused by epidermal hyperplasia or oedema, and was not associated with excess mucus production. Often no causal agent could be seen microscopically, however in some cases a low number of *Ichtyobodo necator* or *Gyrodactylus* spp. could be found, and in fish with ulcers (most probably secondary infections) *Flavobacterium*-like organisms could be seen. Lesions most often were located to the ventral surface, however in severe cases lesions were extending up the sides and on the dorsal surface. No increased mortality was seen but stress and transportation could give rise to mortalities and further development of the lesions, also in some circumstances during the summer an increased ectoparasitic burden and lower water quality seemed to enhance the severity of the lesions. One farmer, which had recognised the problem for several years, told that lesions healed off with decreasing water temperatures during fall and winter. In cases with increased parasitic burden formalin treatment were tried with some effect. Feed with an increased nutritional value, e.g. extra vitamins, or addition of vitamin C also seemed to help lesions to heal. Ferguson *et al.* ('No-mucus skin disease' of rainbow trout, *Oncorhynchus mykiss* (Walbaum): a case report. *J. Fish Dis.* 1995,18,49-57) reported a phenomenon similar to above described which they associated with adhesion of bacteria to the junctions of the individual epidermal cells.

In Wales and Scotland a condition called Red Mark Syndrome with more severe skin lesions have been reported. Lesions are characterized by extended haemorrhages and raised scales. The lesions seem to extend down into the dermis and underlying fat and muscle, sometimes with ulceration but normally an unaffected epidermis is seen. The syndrome has been seen in cold water (below 16 °C). No causal organism has been identified but epidemiological and laboratory data suggest an infectious agent, also lesions seem to heal of with broad antibiotic treatment. An association with *Flavobacterium psychrophilum* or a hypersensitivity reaction has been proposed by Pond (Red Mark Syndrome/Cold Water Strawberry Disease Workshop, Bristol, 13th september 2006. FINFISH NEWS Number 3, 27-28 (Winter/Spring 2007)).

SURVEILLANCE OF HEALTH STATUS ON EIGHT MARINE RAINBOW TROUT FARMS IN DENMARK IN 2006

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The health status of eight marine rainbow trout farms were followed during a period from mid June to mid September 2006. Five to ten fish – newly dead fish, if available – were sampled approximately once every two weeks – in some farms less frequently – for bacteriological and virological investigation. No fish pathogenic viruses were detected, but several fish pathogenic bacteria were found on all farms, and all farms experienced disease and mortality during the sampling period due to bacterial infections. *Yersinia ruckeri* was found on 4 and *Renibacterium salmoninarum* on 5 of the farms, but only during the first part of the surveillance period. This indicates that the fish brought the infection with them from fresh water, and were able to clear the infection during the period in salt water. *Aeromonas salmonicida* subsp. *salmonicida* caused mortality on 5 of the 8 farms, but in contrast to *Y. ruckeri* and *R. salmoninarum*, it persisted throughout the sampling period. Although *Aeromonas salmonicida* subsp. *salmonicida* was probably also brought with carrier fish from fresh water, the fish were not able to clear the infection in the sea. *Vibrio anguillarum* caused mortality on 6 of the 8 farms during the whole sampling period. The majority of the isolates belonged to serotype O1. An interesting observation was the frequent detection of *Photobacterium damsela* subsp. *damsela* in several farms, in particular during the warmest period. The significance of this bacterial species as fish pathogen in Denmark certainly deserves further investigation. During the period with the warmest water temperatures, *Vibrio parahaemolyticus* and *Vibrio vulnificus* were found associated with dead fish in 5 and 2 farms, respectively, however, their role as primary pathogens may be somewhat questionable. *V. vulnificus*, which may be a human pathogenic bacterium, has not previously been associated with rainbow trout in Denmark or elsewhere. Interestingly, both percent mortality during the observation period and frequency of antimicrobial treatment as considerably lower in vaccinated than in unvaccinated fish.

BATH VACCINATION OF RAINBOW TROUT AGAINST *YERSINIA RUCKERI* : EFFECTS OF TEMPERATURE ON PROTECTION AND GENE EXPRESSION

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Protection of rainbow trout fry following bath vaccination with a bacterin based on *Y. ruckeri*, the bacterial pathogen causing enteric red mouth disease (ERM), was investigated at 5, 15 and 25° C. Rainbow trout fry were acclimated for 8 weeks at the three temperatures before vaccination. They were subsequently challenged with *Y. ruckeri* and eight weeks post-vaccination which demonstrated a significant protection of vaccinated fish kept at 15° C. There was no protective effect of vaccination in rainbow trout reared at 5 and 25° C. Spleen tissue was sampled from vaccinated and control fish at 0, 8, 24 and 72 hours post-vaccination in order to analyse gene transcript profiles using quantitative real-time RT-PCR (q-PCR). Gene expression in fish vaccinated at 15° C (the protected fish) was regulated with regard to the pro-inflammatory cytokines INF- γ , TNF- α , IL-6 and the anti-inflammatory cytokines IL-10 and TGF- β , the cell receptors TcR, CD8 α , CD 4, C5aR and the teleost specific immunoglobulin IgT. Immunisation using transfer of plasma from vaccinated fish to naïve fish conferred no protection. This indicates that humoral factors such as Ig and complement are less important in the protection induced by bath vaccination. Expression of cellular factors such as CD8 α was significantly increased in the protected trout and this suggests that cellular factors including cytotoxic T-cells could play a role in immunity against *Y. ruckeri*.

WELFARE OF FARMED FISH FROM HARVEST TO KILLING – MEETING THE FUTURE CHALLENGES

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From 01.07.2008 legislation demanding that farmed fish shall be transported, sedated and killed humanly cost-effective in Norway. There will in other words be a demand for euthanasia (painless killing). The industry must facilitate this demand, while maintaining or increasing their harvesting capability, maintaining or increasing quality of the fish product and decreasing the cost of production. Choice of stunning and killing method is therefore essential.

The most common stunning method for Atlantic salmon is to live chill the fish with or without supplement prior to exsanguination, with or without an additional stunning method. It is generally agreed that live chill CO₂ does not render the animal unconscious, but is instead experienced as highly stressful for the fish. In the decade several other stunning methods have been commercially tested in the salmon industry. One of these is electrical stunning which is now becoming a well established method. Early rigor is, however, still a problem with electrical stunning. It has also been shown that approximately 1 % of the fish regain consciousness before gutting as the wound in the gills clot during exsanguinations. It is, however, interesting to note that death can be achieved by electrical stunning if it is immediately followed by some killing method, for decapitation or percussive stunning. Developing an electrical stunning procedure that minimises pain and meets the requirements of the industry is one of the main goals of the FAREWELL-project.

An important tool in this work is automatic image analysis. This methodology is for instance used to measure colour of fillets, quantify fat distribution, detect melanin spots and identify blood residues. It is also a goal of automatic image analysis to ensure that the fish enter the different electrical stunning and killing machines correctly. This is especially important for percussive stunning devices. Studies have shown that between 20-30% of the fish are not hit correctly, the fish are either too small or too large according to the setting of the machine, consequently do not lose consciousness. Automatic image analysis has the potential to adjust the settings of the machine according to the size and shape of each individual fish.

FIELD-TESTING OF A DNA-VACCINE AGAINST VHS IN RAINBOW TROUT: FINAL RESULT PERSPECTIVES

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Viral haemorrhagic septicaemia (VHS) or Egtved Disease is a serious rhabdovirus infection in farmed rainbow trout in Europe and outbreaks of the disease may result in very high mortalities among fish of all sizes. At present the possible means of control of the disease is stamping out of infected farms in combination with intensive surveillance and control programmes. To prevent spread of the pathogen within the EEC, there are strict restrictions between zones where VHS-virus (VHSV) is detected and zones that are free of the pathogen. The severe losses caused by VHS in farmed fish have led to attempts to develop a vaccine over the past 30 years, including live attenuated virus or recombinant proteins. Some of these vaccines conferred protection under controlled laboratory conditions, but failed or gave variable results in field trials and at present no internationally licensed vaccine for VHS is available.

Comment:

About 10 years ago, a DNA-vaccine based on a plasmid with the gene encoding the viral glycoprotein (G) inserted was developed and has repeatedly been proved to confer good protection of rainbow trout against challenge with VHSV under enclosed experimental conditions. The protection includes an early, non-specific phase, as well as a specific and long-lasting phase. As the vaccine appeared to give highly reproducible results under controlled conditions, the Danish Medical Agency ("Lægemiddelstyrelsen") and several other governmental authorities applied for permission to perform a field-testing of the vaccine. The study including the field trial was performed from 2003-2006.

Rainbow trout fry or fingerlings were vaccinated during the winter months at 2 farms without viral disease. They were then transferred to small net-pens on farms with outbreaks of VHS during the spring 2005 and 2006. The study included safety aspects of the DNA-vaccine such as persistence of the vaccine in vaccinated fish, spread of the vaccine to the environment, uptake of the vaccine in a possible consumer (mink) as well as studies of the immune response following vaccination and following challenge in vaccinated and control fish. The results of VHSV-exposure in the net-pens were very variable from one cage to another, mainly due to outbreaks of other diseases or poor environmental conditions in some of the cages. However, a clear-cut protective effect was observed in some cages supporting an applied potential. Detectable levels of injected vaccine disappeared within a few months after vaccination and no negative side effects were observed among the vaccinated fish.

TEMPERATURE-DEPENDENT EXPRESSION OF IMMUNE-RELEVANT GENES IN RAINBOW TROUT FOLLOWING *YERSINIA RUCKERI* VACCINATION

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The gene expression of immune relevant genes in rainbow trout following vaccination with a bacterin of *ruckeri*, a bacterial pathogen causing enteric red mouth disease (ERM), was investigated at 5, 15 and 25° C. Fish trout were immunized by i.p. injection of a water based *Y. ruckeri* (serotype O1) bacterin and gene expression profiles were compared to control groups injected with phosphate buffered saline (PBS). Blood and tissue (spleen and head-kidney) were taken for subsequent analysis using solid phase enzyme-linked immunosorbent assay (ELISA) and real-time PCR, respectively. The up-regulation of cytokine genes was generally faster and higher at high water temperature with major expression at 25° C. The pro-inflammatory cytokine IL-1 β and IL-6 were significantly up-regulated in all immunized groups whereas the cytokine IL-10 was merely up-regulated in fish at 15 and 25° C. The gene encoding the C5a (anaphylatoxin) receptor was expressed at a significantly increased level in both head-kidney and spleen of immunized fish. The secreted IgM encoding gene was significantly up-regulated in the head-kidney of immunized trout reared at 25° C, and a positive correlation ($r: 0.663$) was found between expression of secreted IgM in the head-kidney and *Y. ruckeri* specific antibodies in plasma measured by ELISA. However, no regulation of the teleost specific immunoglobulin IgT, which was generally expressed at a much higher level than IgM, could be detected. The study indicated that expression of both innate and specific adaptive response genes are highly temperature-dependent in rainbow trout.

SEASONAL DYNAMICS OF *DACTYLOGYRUS* SPP. (MONOGENOIDEA) FROM THE GILLS OF BARB, *PUNTIUS SOPHORE* IN THE RIVER GOMTI, LUCKNOW (INDIA)

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There was a significant increase in number of parasites during summer when compared to numbers in winter. This was explained by the combined action of seasonal differences in temperature and a close adaptation between the different stages of the life cycle of the parasite and its host. A positive correlation existed between parasite infestation and reduced level of dissolved aquatic oxygen. This is because the higher ventilatory water flow, a function of higher levels of dissolved oxygen of the water, results in an increased probability of oncomiracidia containing water over the gills.

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