International Conference on Fish Diseases and Fish Immunology

In Celebration of the 60th Anniversary of the Institute for Experimental Pathology, University of Iceland, Keldur

Venue: Radisson SAS Hotel Saga in Reykjavík, Iceland

Organized by
The Institute for Experimental Pathology, University of Iceland, Keldur
(www.Keldur.hi.is)

Organizing committee
Sigurdur Ingvarsson, chair
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Bjarnheiður K. Gudmundsdóttir
Matthías Eydal

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Welcome from the director of the Institute for Experimental Pathology, University of Iceland, Keldur

In the autumn of the year 1948 the Institute for Experimental Pathology, University of Iceland, Keldur, was established and is therefore celebrating its sixty years anniversary.

From the early days of the institute it has been acting as a university institution focusing on academic veterinary and biomedical research. The major activities have been on animal diseases. The focus has also been on applied veterinary research and diagnostic services.

Research on virology has been performed from the initial years and this resulted in the first description of a lentivirus, the MVV, a pioneering work by Björn Sigurdsson and collaborators. Today the work is still focused on MVV and other viruses, on prions, bacteriae and parasites. These infectious agents are studied by several disciplines; prionology, virology, bacteriology, parasitology, immunology, pathology, molecular biology etc.

For the last three decades there has been remarkable developments at the host institute in the research field of fish diseases and fish immunology and there are ongoing experiments of interest. Therefore it was decided to organize the International Conference on Fish Diseases and Fish Immunology which will take place in Reykjavik Iceland from September 6 – 9, 2008. The conference is arranged as a 60th anniversary of the host institute.

The understanding of infectious fish diseases and the causative agents, like viruses, bacteria and parasites, has been growing fast lately in the international scientific society. In parallel there is an increasing knowledge on host-pathogens interaction, including fish immunology and genome analysis of the pathogens and several fish species. Healthy fish in a clean environment is fundamental for successful fisheries and fish farming industries.

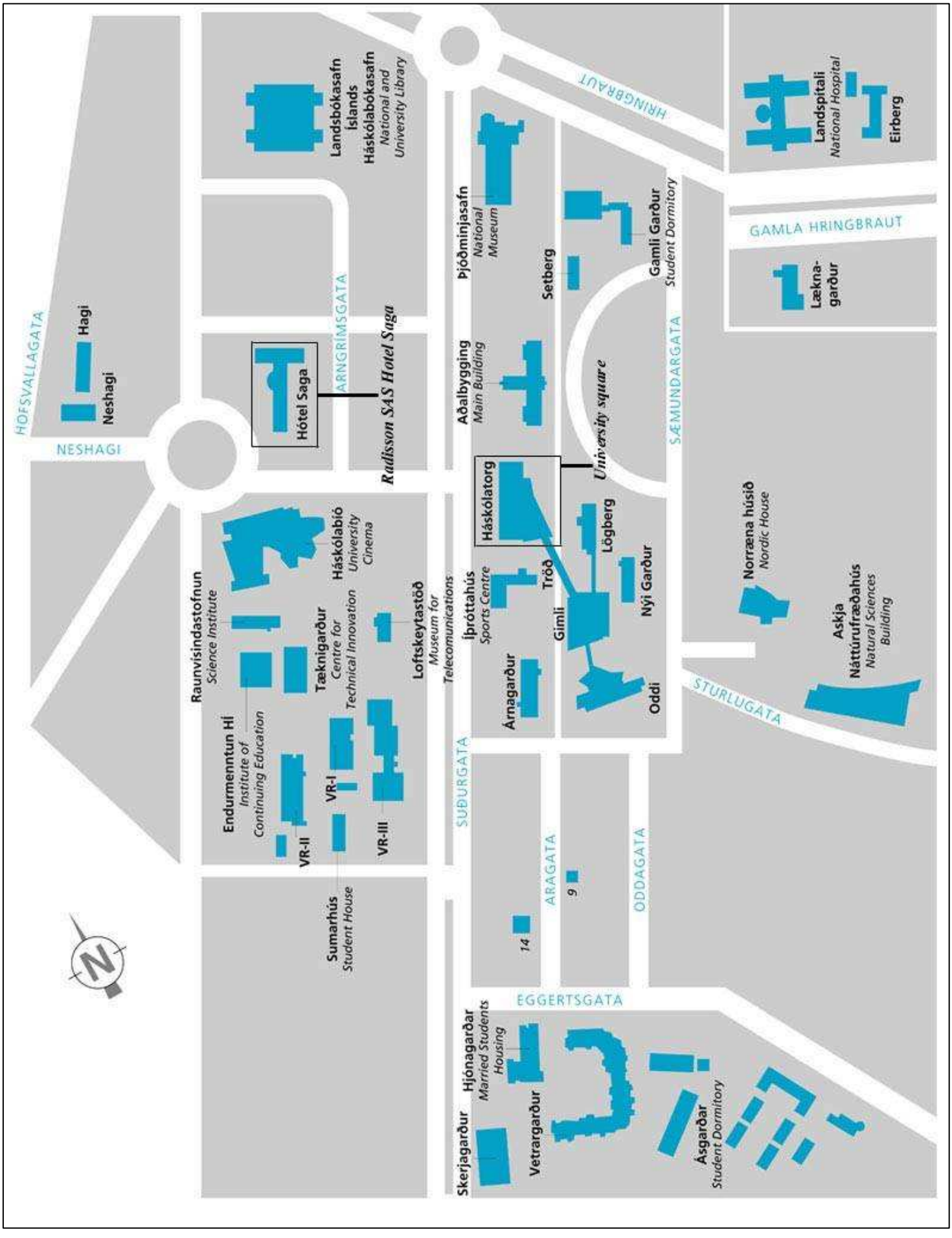
There is an excellent international attendance to the conference from eminent scientists and researchers. I hope that the dynamic spirit of Iceland will provide a conducive milieu in which stimulating scientific exchange can take place amongst scientists from all over the world. I also hope that through this meeting, stronger research ties between the scientists of different regions can be fostered. The event will provide an exclusive platform to discuss the recent developments in the field. I trust that you will enjoy the conference and Iceland, and I look forward to meeting with you during the conference.

I like to thank my co-workers in the organizing committee of the conference, Bjarnheidur K. Gudmundsdottir, Matthias Eydal and Arni Kristmundsson, and to congratulate the scientific committee, Eva Benediktsdottir, Sven M. Bergmann and Bjarnheidur K. Gudmundsdottir for putting together an exciting agenda. I also thank Inga Solnes and Helga Gunnur Thorvaldsdottir at the conference secretariat and those who are financially supporting the conference.

I also want to congratulate and thank all of those who are presently working or have been working in the past at the institute. These are the people who have made the several lasting contributions over the last 60 years, resulting in the progress of the institute into a modern international laboratory in biomedicine and veterinary science, with a strong tradition. Finally, I wish the Institute for Experimental Pathology, University of Iceland, Keldur continued success for at least the next 60 years.

Sigurdur Ingvarsson, director and professor

MAP OF THE UNIVERSITY AREA



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GENERAL INFORMATION

Congress venue

All scientific sessions are held in **Harvard hall** at the Radisson SAS Hótel Saga.

Language

The official language of the conference is English. Presentations and discussions will be conducted in English.

Preparation for oral presentations

All speakers are asked to present themselves with their contributions on CD or USB memory stick not later than during the last break before their presentation.

Posters

The posters will be located in **Yale hall** at the Radisson SAS Hótel Saga. All posters should be mounted on Sunday morning Sept. 7th and removed at the end of the conference. Presenting authors in **Session I** are requested to stand by their posters between 13:30 – 14:30 on Sunday Sept. 7th and authors in **Session II** between 11:00 – 12:00 on Tuesday Sept. 9th.

Lunch and Coffee

Coffee and lunches are included in the registration fee.
Lunch is served in **Sunnusalur** at the Radisson SAS Hotel Saga.

Registration and information

Opening hours for registration and information are as follows:

University square	**Saturday, Sept. 6th**	17:30-20:00
Radisson SAS Hótel Saga	**Sunday, Sept. 7th**	08:00-09:00 onwards

FEMS YSMG GRANTEES

Eva Elisabeth Högfors, PhD student, Åbo Akademi University, Laboratory of Aquatic Pathobiology, Biocity, Tykistökatu 6, 20520 Åbo/Turku, Finland e-mail: eva.hogfors@abo.fi
Date of birth 8th May 1979
FEMS Member Society to which she is subscribed: Societas biochemical, biophysica et micobiologica Fenniae.
Title of an oral presentation in Session 3:
Characteristics of rough and smooth colony types of *Flavobacterium psychrophilum*

Martha R. Becerril, PhD student, Centro de Investigaciones Biologicas del Noroeste S.C., México. Address Sierra Vista y Obrera Mundial #355, Col. Loma Linda. CP. 23030, La Paz B:C:S: México.
Date of birth 30th April 1978
FEMS Member Society. Recommended by Patricia Diaz Rosales, Sociedad Espanola de Microbiologia.
Title of an oral presentation in Session 1:
Immune response in gilthead seabream *Sparus aurata* induced by a potential probiotic live yeast *Debaryomyces hansenii* CBS 8339

Rajeev Singh, PhD student, Lab. No 107, Department of Zoology, University of Delhi, 110007 Deli, India.
Date of birth 10th August 1983
FEMS Member Society. Recommended by Bjarnheidur K. Gudmundsdóttir a member of SGM and the Microbiological Society of Iceland.
Title of an oral presentation in Session 1:
Role of opioid peptides in regulation of splenic phagocyte activities in teleost fish *Channa punctatus*: An *in vitro* study

Tharangani K. Herath, PhD student, Institute of Aquaculture (IoA), University of Stirling, Stirling, UK, FK9 4LA
Date of birth 25th July 1974
FEMS Member Society. Recommended by Bjarnheidur K. Gudmundsdóttir a member of SGM and the Microbiological Society of Iceland
Title of an oral presentation in Session 2:
Alternative Cell Lines for Salmon Alphavirus -1 Isolation
Title of a poster presentation:
Apoptosis Induced Cell Death in Salmonid Alphavirus Infection

These four students will all give an oral presentation at the meeting and one will also present a poster. They get 600€ each for travelling and accommodation plus a free excursion to the Blue Lagoon (102€) and free registration (417€).

SOCIAL PROGRAM AND EXCURSIONS

Saturday, Sept. 6th	
18:00 - 20:00	**Welcoming reception at the University square (Included in registration fee)**

Sunday, Sept. 7th	
19:30 -	**Reception/Dinner - Radisson-SAS-Hotel Saga - Sunnusalur**

Excursions

The following excursions can be arranged by Gestamottakan -Your Host in Iceland. More specific schedule will be announced later. For further information contact the conference organizer yourhost@yourhost.is
Most excursions require a minimum number of participants. Other excursions are available upon request.

Monday, Sept. 8th

17:30 – 22:30 The Blue Lagoon – Bath and dinner

BLUE LAGOON - Geothermal Seawater is a part of a unique eco-cycle where high technology and nature work in perfect harmony in Iceland's environment, and making the BLUE LAGOON - Geothermal Seawater, well-known for its active ingredients and healing power. The origin of the seawater is from 2000 meters beneath the surface. In its travels through porous lava, a blend of sea and fresh water undergoes mineral exchange and then near the surface, concentration occurs, due to vaporization, evaporation and finally, sedimentation. The lagoon at BLUE LAGOON - Geothermal Spa holds four million liters of water, all of which is renewed in 24 hours. The geothermal seawater's special ecosystem has a self-cleansing effect. Regular sampling shows that "common" bacteria do not thrive in this ecosystem. Thus additional cleansers such as chlorine are not needed. Its unique active ingredients: minerals, silica and blue green algae have been praised for their benefits on healthy skin and on skin problems such as eczema and psoriasis. The aquamarine blue colour is the result of these active ingredients: minerals, silica and algae.

Departure at 17:30
Duration approx. 5 hours
Price EUR 102 - Included: Transfer, towel and dinner at the Blue Lagoon Restaurant

Tuesday, Sept. 9th

17:00 – 22:00 Horseback riding on the Outskirts of Reykjavik

The Icelandic Horse is a small but strong, friendly, willing and docile animal who rarely gives riders any trouble. This trip is an excellent trip for all those interested in trying the Icelandic horse, a trek on the hardy, sure-footed Icelandic horse is an experience to remember. Whether you are a beginner or expert, old or young, these friendly horses, with their alert and willing nature are indeed a pleasure to ride in their natural surroundings. Pick up at the Hotel to the farm located around 20 minutes from Reykjavik. At the farm you are provided will all basic equipment, such as helmets, boots and rain clothes. We gather information about how much you have been riding and choose the proper horse to match your experience. A wholesome Icelandic meat soup will be served after the riding at the farm.

Departure at: 17:00 (pick up at 16:30)
Duration around 5 hours
Price: EUR 58

Tuesday, Sept. 9th

17:00 – 21:00 Whale watching

Join us in an adventure at sea with an unforgettable trip into the world of whales and sea birds. The various types of whales commonly sighted include minke whales, white-beaked dolphins, harbour porpoises, the popular humpback whales and killer whales (orcas). The majestic fin- and sei whales are also occasional companions to our boats. We will be accompanied by sea birds such as gannets, puffins, guillemots, cormorants, gulls, kittiwakes, arctic terns, and many more. Our trips take us past several islands inhabited by colonies of puffin.

Departure at: 17:00 (pick up at 16:30)
Duration around 4 hours
Price: EUR 55

Wednesday, Sept. 10th

9:00 – 18:00 Super jeep safari tour - Thorsmork

Heading for Thorsmork valley, a beautiful nature reserve situated between three glaciers. This magnificent valley offers a unique composition of glaciers, rocks and greenery, not to forget the unbridged glacial rivers we have to cross to get there. On the way we will try out the black sands on the south coast if conditions allow and stop at a glacial lagoon by the glacier Gígjökull. When we arrive in Þórsmörk we will be doing some hiking i.e. into Stakkholtsgjá fissure. Note: Wear warm clothes and sturdy shoes!

Pick-up between 08:30 - 09:00
Duration approx. 8 - 10 hours
Price: EUR 232

Wednesday, Sept. 10th

9:00 – 17:00 The Golden Circle – An All Time Classic

The Golden Circle is without doubt one of the most popular tourist destinations in Iceland, and not without reason. The tour consists of the Gullfoss waterfall, the Geysir hot springs and Þingvellir national park, all of which are famous in their own right, but can easily be enjoyed in the course of one day, making for an ideal day tour. Gullfoss, or the "Golden Waterfall" as the name implies, is located on the Hvitá river. The falls cascade 32m down in two stages. Often, colourful rainbows created by the sun on the spraying water can be enjoyed. In winter, massive ice formations decorate the falls. Not to be missed by anyone, the beauty of this waterfall can hardly be expressed fully in words. The geothermal field in Haukadalur is a natural wonder of hot springs and boiling mud pools. The "Great Geyser" even gave its name to this spectacular phenomenon. Today, the Geysir itself is no longer erupting, but nearby Strokkur sends up a column of water and steam up to 30 meters high every few minutes to the delight of onlookers, who strive to catch the moment on film. At the Geysir centre visitors can enjoy a multimedia exhibition and learn more about the geology and history of the area. Also on spot is a restaurant and accommodation for those wishing to stay overnight. In summertime, the highland route of Lyngdalsheiði provides a convenient shortcut to Þingvellir for those traveling on four wheel drive. A UNESCO World Heritage site, Þingvellir national park is of immense historic and symbolic importance to Icelanders. It was long the site of the original Althing, or national parliament of the settlers, and the setting for many of the most important events in the history of the island. Established in 930, the Althing was an assembly of free men that gathered at Þingvellir for two weeks each summer to settle disputes, set laws and arrange marriages. Þingvellir is also renowned for its geological significance. The area is located on the Mid-Atlantic ridge, where the continents of Europe and America drift apart, causing earthquakes and volcanic activity. Standing in the Almannagjá fissure, the visitor is literally situated between the continental plates. Þingvellir is also known for its exquisite beauty. The birch-covered lava fields and the clear blue waters of Lake Þingvallavatn produce a harmonious, almost serene landscape.

Departure at: 09:00
Duration approx. 8 hours
Price: EUR 83

CONFERENCE TIME SCHEDULE

Saturday, Sept. 6th

University Square

17:30 - 20:00	Registration
18:00 – 20:00	Welcoming reception

Sunday, Sept. 7th

Radisson-SAS-Hotel Saga

08:00 - 09:00	Registration
09:00 - 09:15	Opening ceremony
09:15 - 10:00	Plenary lecture
10:00 - 12:30	Session 1: Fish immunology
12:30 - 13:30	Lunch
13:30 - 14:30	Poster session I
14:30 - 17:00	Session 2: Viral diseases of fish and causative agents
19:30	Social event – Reception/Dinner At Radisson-SAS-Hotel Saga – Sunnusalur

Monday Sept. 8th

Radisson-SAS-Hotel Saga

09:00 - 12:30	Session 3: Bacterial diseases of fish and causative agents
12:30 - 13:30	Lunch
13:30 - 15:30	Session 4: Fish parasitology
17:30 -	Social event-Excursion to the Blue Lagoon

Tuesday Sept. 9th

Radisson-SAS-Hotel Saga

09:00 - 11:00	Session 5: NordForsk Session: Communication networks in marine bacteria
11:00 - 12:00	Poster session II
12:00 - 13:00	Lunch
13:00 - 16:45	Session 6: Fish health and prophylaxis
16:45	Closing of the conference

SCIENTIFIC PROGRAM

Saturday, Sept. 6th

University Square

17:30 - 20:00 Registration
18:00 - 20:00 Welcoming reception
18:15 - 18:45 Fish farming in Iceland
Bernharð Laxdal, *Lífsgleði ehf. Iceland*

Sunday, Sept. 7th

Radisson-SAS-Hotel Saga

08:00 - 09:00 Registration
09:00 - 09:15 Opening ceremony
Sigurdur Ingvarsson, *director of the Institute for Experimental Pathology, University of Iceland, Keldur*

Plenary lecture
09:15 - 10:00 Molecular interaction between fish pathogens and host aquatic animals
Laura Brown, *Fisheries and Oceans, Nanaimo, Canada*

10:00 - 12:30
Session 1: Fish immunology
Chair: **Takashi Aoki**, *Tokyo University of Marine Science and Technology*

KN-1: Keynote lecture
10:00 - 10:30 The immune system of cod (*Gadus morhua*)
Bergljot Magnadóttir, *Institute for Experimental Pathology, University of Iceland, Keldur*

10:30 - 11:00 Coffee

O-1
11:00 - 11:15 Cloning and expression analysis of striped trumpeter (*Latris lineata*), pro-inflammatory cytokine genes in response to the ectoparasite *Chondracanthus goldsmidi*
Jen M. Covello, *University of Tasmania, Australia*

O-2
11:15 - 11:30 Genomic tools for cod immunological research: Characterization and expression of CC chemokines of the Atlantic cod (*Gadus morhua*)
Stewart C. Johnson, *Fisheries and Oceans, Nanaimo, Canada*

O-3
11:30 - 11:45 Disease related tissue damage and subsequent changes in fillet structure
Hans-Christian Ingerslev, *Technical University of Denmark*

O-4
11:45 - 12:00 The seasonal changes in immunocompetence of common carp (*Cyprinus carpio*) and the potential associations to the metazoan parasites
Karolína Lamková, *Faculty of Science, Masaryk University, Czech Republic*

O-5
12:00 - 12:15 The antimicrobial peptide cathelicidin in divergent fish species
Valerie H. Maier*, Institute for Biology, University of Iceland*

O-6
12:15 - 12:30 Role of opioid peptides in regulation of splenic phagocyte activities in teleost fish *Channa punctatus*: An *in vitro* study
Rajeev Singh, *Department of Zoology, University of Delhi, India*

12:30 - 13:30 Lunch

13:30 - 14:30 Poster session I

14:30 - 17:00
Session 2: Viral diseases of fish and causative agents
Chair: ***Sigríður Guðmundsdóttir****, Institute for Experimental Pathology, University of Iceland, Keldur*

KN-2: Keynote lecture
14:30 - 15:00 Viral diseases of fish and causative agents
Sven M. Bergmann, *Friedrich-Loeffler-Institut, Insel Riems, Germany*

O-7
15:00 - 15:15 Transcriptomic analysis of Atlantic salmon (*Salmo salar*) head kidney cells infected with infectious pancreatic necrosis virus
William G. Starkey, *Institute of Aquaculture, University of Stirling, Scotland*

O-8
15:15 - 15:30 Persistent nodavirus infection in adult clinically healthy cod *Gadus morhua*
Mona Gjessing, *National Veterinary Institute, Norway*

15:30 - 16:00 Coffee

O-9
16:00 - 16:15 Alternative cell lines for salmon alphavirus -1 isolation
T. K. Herath, *Institute of Aquaculture, University of Stirling, Scotland*

O-10
16:15 - 16:30 Infectious salmon anaemia virus HPR0, surveillance in marine Atlantic salmon farms across Scotland
Alastair McBeath, *Fisheries Research Services, Scotland*

O-11
16:30 - 16:45 Antibody response of the Atlantic salmon (*Salmo salar* L.) against a soluble form of the ISAV hemagglutininesterase
Bjarne Reinert, *Norwegian College of Fishery Science/University of Tromsø, Norway*

O-12
16:45 - 17:00 Gene expression and viral infection studies of Atlantic cod cells
Marit Seppola, *Nofima Marin, Norway*

Monday Sept. 8th

Radisson-SAS-Hotel Saga

09:00 - 12:30
Session 3: Bacterial diseases of fish and causative agents
Chair: ***Helene Mikkelsen***, *Nofima Marine Norway*

KN-3: Keynote lecture
09:00 - 09:30 Diagnosis and management of bacterial kidney disease: Present and future
Diane G. Elliott, *Western Fisheries Research Center, USGS, USA*

O-13
09:30 - 09:45 Epidemiology of *Renibacterium* salmoninarum in farmed salmonids in Iceland
Árni Kristmundsson, *Institute for Experimental Pathology, University of Iceland, Keldur*

O-14
09:45 - 10:00 *Renibacterium salmoninarum*: A novel PCR and isolation of DNA from samples
Ívar Ö. Árnason, *Institute for Experimental Pathology, University of Iceland, Keldur*

O-15
10:00 - 10:15 Fish tuberculosis - A case study of host/pathogen interaction
Sonia Dias, *Universidade do Porto, Portugal*

O-16
10:15 - 10:30 Experimental mycobacteriosis in Atlantic cod, *Gadus morhua*
Mulualem A. Zerihun, *National Veterinary Institute, Oslo, Norway*

10:30 - 11:00 **Coffee**

Chair: ***Eva Benediktsdóttir****, Institute for Biology, University of Iceland*

O-17
11:00 - 11:15 Characteristics of rough and smooth colony types of *Flavobacterium psychrophilum*
Eva Högfors, *Åbo Akademi University, Laboratory of Aquatic Pathobiology, Finland*

O-18
11:15 - 11:30 Diversity with in the *Francisella philomiragia* group
Jarle Mikalsen, *National Veterinary Institute, Oslo, Norway*

O-19
11:30 - 11:45 Challenge models for effluent mediated transmission of classical vibriosis and atypical furunculosis between fish species
Helene Mikkelsen, *Nofima Marine, Norway*

O-20
11:45 - 12:00 *Moritella viscosa* subsp. *iridensis*, a "new" pathogen of farmed rainbow trout in Norway
Duncan J. Colquhoun, *National Veterinary Institute, Oslo, Norway*

O-21
12:00 - 12:15 Real-time PCR detection of the winter ulcer bacterium *Moritella viscosa* and immunogenic responses after bath challenge of Atlantic salmon
Marie Løvoll, *National Veterinary Institute, Oslo, Norway*

O-22
12:15 - 12:30 Characterisation and virulence assay of an extracellular vibriolysin of *Moritella viscosa*
Bryndís Björnsdóttir, *Institute for Experimental Pathology, University of Iceland, Keldur*

12:30 - 13:30 **Lunch**

13:30 - 15:30
Session 4: Fish parasitology
Chair: ***Arne Levsen****, National institute of nutrition and seafood research (NIFES), Norway*

KN-4: Keynote lecture
13:30 - 14:00 Parasites causing disease in wild and cultured fish in Newfoundland
Rasul A. Khan, *Memorial University of Newfoundland, Canada*

O-23
14:00 - 14:15 *Loma morhua* infections in Atlantic cod (*Gadus morhua*):
A molecular diagnostic assay for the elucidation of epidemiological factors during aquaculture
A. P. Frenette, *University of New Brunswick, Canada*

O-24
14:15-14:30 Red vent syndrome (RVS) in wild Atlantic salmon in Scotland
Noguera Patricia, *FRS Marine Laboratory, Scotland*

O-25
14:30 - 14:45 Parasites of resident arctic charr, *Salvelinus alpinus,* and brown trout, *Salmo trutta,* in two lakes in Iceland.
Sigurður H. Richter, *Institute for Experimental Pathology, University of Iceland, Keldur*

O-26
14:45 - 15:00 Immunological parameters and parasite infection in chub *Leuciscus cephalus* in heavily polluted watershed
M. Ondrackova, *Institute of Vertebrate Biology ASCR, Czech Republic*

O-27
15:00 - 15:15 Stress response in rainbow trout during infection with *Ichthyophthirius multifiliis* and formalin bath treatment
Thomas R. Jørgensen, *University of Copenhagen, Faculty of Life sciences, Section of Fish Diseases, Denmark*

O-28
15:15 - 15:30 X-cell disease (pseudobranchial tumours) in wild and farmed young cod, *Gadus morhua* L., in Iceland with a direct transmission study
Matthías Eydal, *Institute for Experimental Pathology, University of Iceland, Keldur*

17:30 **Social event -** Excursion to the Blue Lagoon

Tuesday Sept. 9th

Radisson-SAS-Hotel Saga

09:00 -11:00
Session 5: NordForsk Session: Communication networks in marine bacteria
Chair: ***Henning Sørum****, Norwegian School of Veterinary Science*

KN-5: Keynote lecture
09:00 - 09:30 Probiotics for marine fish larvae
Lone Gram, *Technical University of Denmark*

KN-6: Keynote lecture
09:30 - 10:00 Quorum sensing and virulence in *Vibrio anguillarum*
Debra Milton, *Umeå University, Sweden*

O-29
10:00 - 10:15 The RNA chaperone Hfq regulates signal molecule production in *Vibrio anguillarum*
Kristoffer Lindell, *Umeå University, Sweden*

O-30
10:15 - 10:30 Cytotoxic, adhesive and invasive properties of the fish pathogen *Moritella visc*osa
Hege Smith Tunsjø, *Norwegian School of Veterinary Science, Norway*

O-31
10:30 - 10:45 Generation and phenotypic screening of a *Vibrio salmonicida* transposon mutant library
Ane Mohn, *Norwegian School of Veterinary Science, Norway*

O-32
10:45 - 11:00 Whole genome sequencing of *Vibrio salmonicida* as platform to study basic mechanisms in virulence
Peik Haugen, *University of Tromsø, Norway*

11:00 - 12:00 **Poster session II**

12:00 - 13:00 **Lunch**

13:00 - 16:45
Session 6: Fish health and prophylaxis
Chair: ***Stewart Johnson****, Fisheries and Oceans, Canada*

KN-7: Keynote lecture
13:00 - 13:30 Diseases of cold water fish species
Øivind Bergh, *Institute of Marine Research, Norway*
O-33
13:30 - 13:45 Improved vaccine against *Moritella viscosa*
Tor Lunder, *ScanVacc AS, Drøbak, Norway*
O-34
13:45 - 14:00 Combined effects of stress and vaccination on the metabolic rate of Atlantic salmon smolts
Mark D. Powell, *Bodø University College, Norway*
O-35
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Kira Salonius, *Novartis Animal Health, Aqua Health Business, Canada*
O-36
14:15 - 14:30 From laboratory to the field: Was the timing right to take an experimental AGD vaccine to sea?
Mathew T. Cook, *CSIRO Food Futures Flagship, CSIRO Marine and Atmospheric Research, Hobart, Tasmania, Australia*
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14:30 - 14:45 Th1 and Th17 responses after *Vibrio anguillarum* vaccination in the gadoid haddock *(Melanogrammus aeglefinus*)
Yolanda Corripio-Miyar, *Scottish Fish Immunology Research Centre, University of Aberdeen, Scotland*
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14:45 - 15:00 Oligonucleotide microarray: An effective platform for antimicrobial resistance genotyping
Takashi Aoki, *Tokyo University of Marine Science and Technology, Japan*

15:00 - 15:30 **Coffee**

15:30 - 16:45 Session 6: Fish health and prophylaxis-continued
*Chair **Lone Gram,** Technical University of Denmark*

KN-8: Keynote lecture

15:30 - 16:00	Persisting effects of different first feeding methods on Atlantic cod juveniles **Albert Imsland,** *Akvaplan-niva and University of Bergen, Norway*
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16:15 - 16:30	Application of putative probionts at larval and juvenile stages of Atlantic Cod (*Gadus morhua* L.) rearing **Hélène L. Lauzon**, *Matis, Iceland*
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16:30 - 16:45	Immune response in gilthead seabream *Sparus aurata* induced by a potential probiotic live yeast *Debaryomyces hansenii* CBS 8339 **Martha R. Becerril,** *Centro de Investigaciones Biologicas del Noroeste S.C., México*
16:45	**Closing of the conference** **Sigurður Helgason,** *Head of Fish Disease Laboratory**,** Institute for Experimental Pathology, University of Iceland, Keldur*

LIST OF ABSTRACTS

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O-5 The antimicrobial peptide cathelicidin in divergent fish species
V.H. Maier, C.N.Z. Schmitt, K. Dorn, B.K. Guðmundsdóttir and G.H. Guðmundsson

O-6 Role of opioid peptides in regulation of splenic phagocyte activities in teleost fish *Channa punctatus*: An *in vitro* study
R. Singh and U. Rai

O-7 Transcriptomic analysis of Atlantic salmon (*Salmo salar*) head kidney cells infected with infectious pancreaticnecrosis virus
William G. Starkey, J.E. Bron, J.B. Taggart, J.H. Ireland, A.E. Ellis, R. Talbot, S.N. Carmichael, A.J. Teale and R.H. Richards

O-8 Persistent nodavirus infection in adult clinically healthy cod *Gadus morhua*
Mona Gjessing, Agnar Kvellestad, Knut Falk and Kristin Ottesen

O-9 Alternative cell lines for salmon alphavirus -1 isolation
T.K. Herath, J.Z. Costa, K.D. Thompson, A. Adams and R.H. Richards

O-10 Infectious salmon anaemia virus HPR0 surveillance in marine Atlantic
A. McBeath, N. Bain and M. Snow

O-11 Antibody response of the Atlantic salmon (*Salmo salar* L.) against a soluble form of the ISAV hemagglutininesterase
B. Reinert, A. Müller, T.Ø. Jørgensen and S.T. Solem

O-12 Gene expression and viral infection studies of Atlantic cod cells
M. Seppola, K. Steiro, S. Mennen, A.I. Sommer, I. Jensen

O-13 Epidemiology of *Renibacterium salmoninarum* in farmed salmonids in Iceland
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ABSTRACTS

Plenary lecture

PL
Molecular interaction between fish pathogens and host aquatic animals

Laura L. Brown and Stewart C. Johnson

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While studying the host-pathogen interactions between Atlantic salmon (Salmo salar L.) and *Aeromonas salmonicida*, we sequenced the genome of the bacterium and investigated gene products with potential as vaccines. Using knockout mutants of *A. salmonicida*, we identified key virulence factors. Proteomics studies of bacterial cells grown in selected media and an in vivo implant system revealed differential protein production and have shed new light on bacterial proteins such as superoxide dismutase, pili and flagellar proteins, type three secretion systems, and their roles in *A. salmonicida* pathogenicity. We constructed a whole genome DNA microarray to use in comparative genomic hybridizations (M-CGH) and bacterial gene expression studies. Carbohydrate analysis showed variation in LPS between strains and reveals the importance of LPS in virulence. Salmon were challenged with *A. salmonicida* and tissues were taken to construct suppressive subtractive hybridization libraries to investigate differential host gene expression. We constructed an Atlantic salmon cDNA microarray to investigate the host response to *A. salmonicida*. Real-Time qPCR and NMR-based metabolomics have revealed important information about host responses to infection and to chronic stress. By linking genome sequencing, functional genomics, proteomics, carbohydrate analysis, metabolomics, and whole animal assays, we took integrated and innovative approach to pathogenesis research.

Key note lectures

KN-1
The immune system of Cod (*Gadus morhua* L.)

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The Atlantic cod, *Gadus morhua* L., is of high economic importance and until recently the mainstay of Iceland's economy. Excessive fishing and climatic changes now threaten cod stocks everywhere. Hence, there is a growing interest in cod farming and experimental and commercial projects have been established in several countries. Cod farming has been relatively uncomplicated but setbacks are caused by losses during larval stages and disease problems, often associated high density rearing. Consequently there has been a growing interest in studies of the immune system and immune defence of cod and of prophylactic measures in cod aquaculture. Most teleost fish produce specific immune response and can be successfully vaccinated against different diseases. Cod, and possibly all Gadoids, is apparently an exception to this rule its specific antibody response being generally poor. This defect is especially interesting since the gadoids belong to the most recent and presumably most advanced group of the teleost species. In spite of this deficiency cod shows effective immune defence and vaccination can result in significant and specific protection. A considerable work has been carried out in various laboratories to try and understand the defence mechanism of cod and how it differs from other fish species. An overview of this groundwork will be the subject of this talk with emphasis on recent studies carried out at the Institute for Experimental Pathology.

KN-2
Viral diseases of fish and causative agents

Sven M. Bergmann

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Over the last 10 years new fish species introduced new disease agents and diseases into the European as well as in the world-wide aquaculture. Only the development of new diagnostical tools allowed the detection of these agents. Diseases and their pathogens, notifiable to European Union (EU) and World Organization for Animal Health (OIE), are well known and mostly detectable by methods as cultivation in permanently growing cell cultures, antigen enzyme-linked immunosorbent assay (antigen ELISA), immunofluorescence assay (IFA) with polyclonal or monoclonal antisera, serum neutralization test (SNT) and, in some cases, polymerase chain reactions (PCR) or reverse transcription PCR (RT-PCR). Unfortunately, serological assays using serum antibodies directly from infected fish are not among the so called allowed diagnostical methods. While most diagnostic laboratories have no problems to use validated and published methods for detection of notifiable infectious agents, new diseases occurred in newly aquacultured species. Instead of well validated diagnostical methods, mostly laboratory or in-house but accredited procedures are used for diagnosis.

Due to the enlargement of the EU, the cyprinid aquaculture production increased enormously since countries as Poland, Czech Republic or Hungary are main producers of these animals. Since 1996, a new disease has occurred called Koi Herpesvirus disease. First recognized by electron microscopy, the agent, which is difficult to isolate in cell cultures, is mostly recognized by PCR. For confirmation of these results only sequence analysis of PCR products, electron microscopy and IFA or *in-situ* hybridization (ISH) on tissue sections are available. An additional problem is the diagnosis of new agents in eel aquaculture. Over the last years picorna-, birna-, herpes-, rhabdo- and reoviruses have been found in sick elvers. It is completely unknown if glass eels caught in the wild already carry these pathogens. It is not known which pathogen is influencing the production process. Also, the world-wide trade with ornamental fish confronted the aquaculture with new problems to the spread of fish and pathogens in natural waters. Examples for these transfers are detections of irido-, othomyxo-, picorna- , reo- and herpesviruses in different aquacultured fish species as well as in ornamental fish. Methods used for identification and characterization of these agents are electron microscopy, IFA with monoclonal antibodies, ISH with different probes, cultivation in different cell lines, SNT, PCR and / or RT-PCR but also new approaches like realtime PCR.

KN-3
Diagnosis and management of Bacterial Kidney Disease: Present and future

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Bacterial kidney disease (BKD) caused by Renibacterium salmoninarum (Rs) occurs in most parts of the world where wild or cultured salmonid fishes exist. BKD can cause serious mortality in juvenile salmonids in both fresh water and seawater, and also in pre-spawning adults. Unique characteristics of Rs and its biology have for many years presented formidable obstacles to development of effective tools for detection of Rs and management of BKD. Included among these obstacles are dual modes of Rs transmission (vertical and horizontal), the chronic nature of infection and intracellular existence of Rs, and the slow and fastidious growth of Rs in culture. It is anticipated that recent advances in Rs research will yield important information about the epizootiology of BKD and increase understanding of critical elements of interactions between Rs and the host fish. Development of molecular tools is continuing for Rs detection and quantification and for investigation of host responses to Rs. The Rs genome has now been sequenced, and current gene characterization studies are focusing on antibiotic resistance factors and potential vaccine candidates. Information gained from this research should be useful for improving methods for prevention and control of BKD.

KN-4
Parasites causing disease in wild and cultured fish in Newfoundland

Rasul A. Khan

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This study, based on laboratory and field observations, investigated the role of parasites as the cause of disease outbreaks and mass mortality in cultured and wild fish in Newfoundland. Two protozoans, *Trichodina jadranica* (Ciliophora) and *Loma branchialis* (Microspora) were responsible for mass mortality of cultured fry and fingerling Atlantic cod (*Gadus morhua*) while a myxozoan, *Tetracapsuloides bryosalmonae*, and plerocercoids of a cestode, *Dipyllobothrium dendriticum*, caused a die-off of hatchery-reared Arctic charr *(Salvelinus alpinus*) and steelhead trout *(Salmo trutta*) respectively after transfer to outdoor cages. A hematophagous copepod, *Lernaeocera branchialis*, was associated with mortality of wild Atlantic cod both in the field and the laboratory. Five additional parasites caused morbidity in infected fish hosts. These included two protozoans, *Trypanosoma murmanensis*, inducing anemia in American plaice (*Hippoglossoides platessoides*) and swim bladder lesions by *Goussia caseosa* (Coccidia) in a deep-sea grenadier, *Macrourus berglax*. Skin damage was caused by two mongeneans, *Gyrodactylus pleuronecti* and *Entobdella hippoglossi*, infecting winter flounder (*Pleuronectes americanus*) and Atlantic halibut (*Hippoglossus hippoglossus*) respectively and also in winter flounder by metacercariae of a digenean, *Cryptocotyle lingua*. Xenomas of a microsporan, *Glugea stephani*, infested the internal organs of winter flounder living only at a site contaminated with effluent discharged by a paper mill. It is surmised that parasites have played a major role in mortality of both cultured and wild fish in Newfoundland.

KN-5
Probiotics for marine fish larvae

Lone Gram

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Farming of several marine fish is a complex process and the fish are, especially at the larval stages, susceptible to bacterial infections. The production relies on live feed as well as feed for the feed. For instance, the production of turbot will require production of *Artemia*, rotifers, algae and yeast. Crashes of the larval population occur and these may to some extend be controlled by antibiotics. However, there is an intense development towards alternative disease control measures due to concerns about development of antibiotic resistance. Many larval rearing environment carries a natural microbiota that harbours members capable of inhibiting the growth of fish larval pathogens. We have demonstrated that bacteria belonging to the *Roseobacter* clade can be isolated on marine agar at several steps in turbot rearing. These organisms are not pathogenic to the fish larvae and are capable of inhibiting fish pathogenic *Vibrio* spp. in a well-diffusion assay. The majority of inhibitory strains have been identified as either *Ruegeria* spp. or *Phaobacter inhibens*. The main inhibitory compound is

the sulphur-containing tropodithietic acid which is produced under stagnant conditions or when attached to surfaces. The paper discusses isolation and testing of potential probiotic bacteria and out-lines the current short-comings in probiotic strategies. Future research should focus on determining, which *in vitro* characteristics of a bacterium are indicative of *in vivo* effect.

KN-6
Quorum sensing and virulence in *Vibrio anguillarum*

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Vibrio anguillarum is part of the normal flora as well as an opportunistic pathogen of marine fish. In this pathogen, quorum sensing plays a central role in bacterial physiology by modulating adaptation to stress, such as nutrient starvation in seawater. Using In Vivo Imaging Analysis and a rainbow trout model, *V. anguillarum* was shown to colonize the nutrient rich skin surface before colonizing internal organs such as the intestines, spleen or kidney. Scanning electron microscopy showed that *V. anguillarum* forms a monolayer on the scale collagen surface via some type of appendage and prefers to bind within the growth rings. Colonization of the skin surface was controlled by the newly discovered type VI protein secretion system. Mutants blocked in this secretion system colonized the skin surface more efficiently causing increased spreading of the bacterium in the animal Interestingly, VanT, the main gene regulator induced by quorum sensing, inhibited the type VI secretion system. In summary, stress, such as starvation in seawater, likely stimulates quorum sensing to induce VanT expression which in turn represses the type VI secretion system thus stimulating colonization of the skin surface.

KN-7
Diseases of cold-water fish species

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Atlantic cod, *Gadus morhua* and halibut *Hippoglossus hippoglossus* are now established in aquaculture in several countries around the north Atlantic. Losses have been high during larval and juvenile stages, and vibriosis has long been the most important bacterial disease in cod. However, *Francisella piscicida* (*F. philomeragia* subsp. *noatuniensis*) has now become the most important threat to cod farming. Field studies indicate that the bacterium is widespread in North Sea and along the coast of Southern Norway, but it has not yet been detected in wild cod in Northern Norway. Restrictions on transport of farmed fish have been suggested to avoid negative impact on wild populations from aquaculture. Vaccination of cod and halibut against pathogens such as *L. anguillarum* and *Aeromonas salmonicida* clearly demonstrates that the immune system possesses an effective memory and appropriate mechanisms sufficient for protection, at least against some diseases. Well known viruses such as nodavirus, causing viral encephalopathy and retinopathy (VER), infectious pancreatic necrosis virus (IPNV) and

have been isolated, and can be a potential problem under intensive rearing conditions. In particular, nodaviruses have caused high mortalities in halibut farming, but has also emerged as a problem in cod aquaculture. No commercial vaccines against nodavirus are currently available. Viral haemorrhagic septicaemia virus (VHSV) is a potential problem.

KN-8
Persisting effects of different first feeding methods on Atlantic cod juveniles

Albert Kjartansson Imsland[1], Roland Koedijk[2], Atle Foss[3], Arild Folkvord[2], Sigurd Olav Stefansson[2] and Thor Magne Jonassen[4]

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Intensive feeding schemes are becoming key requirements for successful season-independent intensive Atlantic cod juvenile production. In this presentation recent data from two Norwegian studies will be presented. The two fish groups studied differed by having been start-fed with either rotifers or natural zooplankton, after which they were weaned onto the same commercial diet and otherwise co-reared identical. In the first study we found higher growth (16 %), elevated food intake (20 %) and higher conversion efficiency (22 %) and higher incidence of anatomical deformities in cod juveniles fed rotifers during the larval stage. Growth differences were still persistent following the 30 months of sea-pen ongrowing. In the second study we investigated whether first feeding diet has an effect on the physiological flexibility towards water quality in the juvenile period. The same two groups of juvenile Atlantic cod were exposed to six combinations of ammonia and oxygen for 64 days. Our findings show that cod juveniles startfed with zooplankton have a higher tolerance towards toxic ammonia and hypoxia compared to juvenile cod fed enriched rotifers during the larval period. This suggests that prey type or quality of diet at first feeding may be a determining factor in the development of an adaptive response towards high ambient ammonia and hypoxia during the later juvenile stage.

Oral presentations

SESSION 1. FISH IMMUNOLOGY

O-1
Cloning and expression analysis of striped trumpeter (*Latris lineata*) pro-inflammatory cytokine genes in response to the ectoparasite *Chondracanthus goldsmidi*

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The striped trumpeter (*Latris lineata*) is a new species under investigation for commercial culture in Tasmania, Australia. Although much research effort has gone into understanding reproduction, larval culture, and identification and control of disease, there has been little work focused on understanding the immune response of this species. As the culture of striped trumpeter continues to expand, health issues will inevitably arise, making this area of research increasingly important. Recently, a new species of Chondracanthid parasite with the ability to infect cultured striped trumpeter was described. Preliminary histological examination revealed evidence of an inflammatory response by the host. In light of this discovery, investigation of the level of host response to the parasite on a molecular level was undertaken. To this end, the striped trumpeter IL-1 beta;, IL-8 and TNF-alpha; genes were cloned and sequenced. Real-time, reverse transcription PCR was preformed on gill, head kidney and spleen tissues from parasitized and non-parasitized fish. Results show an up-regulation of proinflammatory cytokines in the parasitized fish compared to the non-parasitized control fish. An understanding of the extent of the host's response will be useful for estimating the level of damage that may occur in the event of a large scale infestation on a population of cultured striped trumpeter. It may also help to elucidate possible mitigation strategies should such an event occur.

O-2
Genomic tools for cod immunological research: Characterization and expression of CC chemokines of the Atlantic Cod (*Gadus morhua*)

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As a part of the Atlantic Cod Genomics and Broodstock Development Project (www.codgene.ca) we are identifying genes involved in both the innate and adaptive immune system of Atlantic cod. Our goal is to use these genes to examine: 1) the immune response of Atlantic cod to viral and bacterial pathogens and 2) how environmental stressors effect immune function. To this end we have generated approximately 154,000 ESTs from normalized full length and subtractive cDNA libraries from both antigen stimulated and non-stimulated tissues. Using BLAST we identified sequences within this database that resemble CC chemokines from fish and higher vertebrates. To date we have identified a total of 26 CC chemokine-like sequences from cod. Phylogenetic analysis places these sequences within 5 of the 7 groups of CC chemokines described in vertebrates. Examination of the tissue distribution of CC chemokines ESTs indicate that most sequences originated from libraries of polyriboinosinic polyribocytidylic acid (pIC)-stimulated kidney, pIC-stimulated spleen, and thermally stressed liver. We are presently examining the expression of select CC chemokines in immune-related tissues following stimulation with the viral mimic polyI:C and formalin-killed atypical *Aeromonas salmonicida*.

O-3
Disease related tissue damage and subsequent changes in filet structure

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Fish meat quality is influenced by many biological and physical factors like e.g. rearing, feeding, slaughtering, processing and storage. Observations from the commercial aquaculture industry indicate that infections in e.g. salmon caused by *Moritella viscosa* or Pancreas Disease (PD) results in downgrading of fish quality and subsequent a reduction in prize. Despite this, the impact of infectious diseases on the meat quality and the mechanisms behind are poorly investigated. Wound repair is a dynamic, interactive response to tissue injury that involves a complex interaction and cross talk of various cell types, extracellular matrix molecules, soluble mediators and cytokines. In order to describe the molecular mechanisms and processes of wound repair, a panel of genes covering immunological factors and tissue regeneration were used to measure changes at the mRNA level following mechanical tissue damage in rainbow trout (*Oncorhynchus mykiss*). Needle disrupted muscle tissue was sampled at different time points and subject to real-time RT-PCR for measuring the expression of the

genes IL-1beta, IL-8, IL-10, TGF-beta;, Myostatin-1ab, MMP-2, CTGF, Collagen-1alpha;, VEGF, iNOS, Arg-2 and FGF. The results showed an initial phase with up-regulation of immune-related genes followed by a regenerative phase with regulation of genes coding for muscle growth and synthesis of connective tissue.

O-4

The seasonal changes in immunocompetence of common carp (*Cyprinus carpio*) and the potential associations to the metazoan parasites

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The immune function of teleost fish is strongly influenced by water temperature. Then, the investment in many fish functions and also the parasite life cycle can vary in time due to the seasonal effects of surrounding water. The aim of this study was to analyze the selected immunological and physiological parameters in *Cyprinus carpio* and investigate the potential links between these parameters and the presence of metazoan parasites. During four different periods from 2007 to 2008 (i.e. early summer, late summer, autumn and winter) a total of 120 individuals of common carp from farmed Vodnany population were studied. Generally, the highest values of majority hematological parameters (RBC, PCV, Hb, MCV, MCH and MCHC) and spleen-somatic index (SSI) were recorded in early summer. The maximal values of lymphocyte and monocyte counts and also lysozyme level were detected in late summer. In autumn, we found the statistically highest values of gonado-somatic index and leukocyte count. Significantly higher values of condition factor (CF), as well values connected with respiratory burst (RB) and phagocyte count (PH) were recorded in winter. The negative correlation was found between CF and the abundance of Monogenea. Moreover, the negative correlation was also found between SSI and both Monogenea and Cestoda. Further, we observed the positive correlation between SSI and both RB and PH.

O-5

The antimicrobial peptide cathelicidin in divergent fish species

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Antimicrobial peptides are at the forefront of immunity and have been shown to be essential in the defence of multicellular organisms against pathogens. One class of antimicrobial peptides are the cathelicidins and they have been extensively studied in mammals. Cathelicidins in mammals have been found to be multifunctional, playing not only a role in antibacterial defences, but also in the recruitment of the adaptive immune system to the site of infection and in angiogenesis. Several cathelicidins have been discovered in salmonids, but so

far little is known about their function in fish. The aim of this study was to identify novel cathelicidins in fish species important in aquaculture in Iceland such as arctic charr and Atlantic cod and to study their expression. We have identified several novel cathelicidins both via Rapid Amplification of cDNA Ends (3´RACE) cloning and database search and compared all discovered fish cathelicidins in a phylogenetic study. We have also studied the tissue expression of cathelicidin in healthy and infected fish and found the expression to increase greatly after exposure of the fish to pathogens. This indicates a role of these proteins in the innate immune response of fish.

O-6
Role of opioid peptides in regulation of splenic phagocyte activities in teleost fish *Channa punctatus*: An *in vitro* study

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The role of opioid peptides in control of immune responses is well studied in mammals. However, least efforts are made in ectothermic vertebrates including fishes. In the present study, the effect of beta-endorphin (beta-end) and methionine-enkephalin (met-enk) in the control of phagocytic and cytotoxic activities of fish phagocytes was investigated. Also, the involvement of specific opioid receptor was explored. beta-end stimulated the phagocytosis, while inhibited the nitrite production. However, it had concentration-related biphasic effects on superoxide production. On the other hand, met-enk stimulated phagocytosis and superoxide, while inhibited the nitrite production. Naltrexone abolished the effect of both beta-end and met-enk on phagocyte functions. Moreover, μ-receptor antagonist CTAP completely blocked the effect of beta-end on phagocytosis and nitrite production. Whereas, stimulatory effect of beta-end on superoxide production at lower concentration was blocked by CTAP and the inhibitor effect at higher concentration was antagonized by δ-receptor antagonist NTI. On the other hand, NTI completely blocked the effect of met-enk on phagocytic and cytotoxic activities of phagocytes. However, the selective μ- and k-receptor antagonists were ineffective in influencing the effect of metenk on any of the function of phagocytes. It appears that beta-end and met-enk regulates the phagocytic and cytotoxic activities of phagocytes through different selective-opioid receptor in *C. punctatus*.

SESSION 2. VIRAL DISEASES OF FISH AND CAUSATIVE AGENTS

O-7

Transcriptomic analysis of Atlantic salmon (*Salmo salar*) head kidney cells infected with infectious pancreaticnecrosis virus

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Infectious pancreatic necrosis virus (IPNV) is a major pathogen of Atlantic salmon. The mechanism by which pathology is induced in IPNV infection is unknown, which compromises breeding programmes aimed at improving disease resistance in Atlantic salmon, and the rational development of vaccines to counter IPN. To characterize the host-pathogen relationship in IPNV, we have analysed transcriptional profiles in IPNV-infected salmon head kidney (SHK-1) cells. RNA was extracted from IPNV-infected SHK-1 cells using the Trizol method and subsequently amplified using the amino allyl MessageAmp procedure. Amplified RNA was Cy3/Cy5 labelled then hybridized to the TRAITS / SGP 17000 feature Atlantic salmon cDNA microarray, which is immune-enriched. At three days postinfection, 340 genes were differentially expressed in IPNV-infected SHK cells. These included genes encoding proteins involved in transcription, innate and adaptive immunity, cellular metabolism, signal transduction, apoptosis, and the cell cycle. Differentially expressed immune-related genes included: NF-kappaB inhibitor, ubiquitin specific protease 18, C-type lectin receptor A, complement factor-H precursor, histamine Nmethyltransferase, proteasome subunit beta type 5, and endothelial leukocyte adhesion molecule (ELAM-1). This work forms part of a larger study which aims to characterize transcriptional expression *in vivo* and which will allow comparisons between in vitro and *in vivo* expression.

O-8

Persistent nodavirus infection in adult clinically healthy cod *Gadus morhua*

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Cod farming industry is growing rapidly. By year 2010, it is estimated that the total production of gadoids will reach approximately 160.000 t, of which Norway will produce 50%. Disease occurs in all farmed fish populations and in the case of cultivation of Atlantic cod, bacterial and parasitic infections apparently have caused the greatest problems. However, viral nervous necrosis (VNN) in cod was reported for the first time in 2001 in Scotland. VNN was detected for the first time in farmed cod in Norway in 2006.Infections with piscine nodavirus have been described in around 30 different marine fish species from all over the world. In commercial aquaculture, VNN is primarily associated with a mass mortality in

larvae. However, infection associated with clinical signs and pathological changes in adult fish has been described in different kinds of groupers and European sea bass, *Dicentrarchus labrax*, whereas in Atlantic halibut, *Hippoglossus hippoglossus*, subclinical infection with pathological changes has been observed. In this study we show that nodavirus infection and related pathological changes can be detected in apparently healthy cod from a farm 14 months after VNN was first detected. Some of the pathological changes described here have never been described earlier.

O-9
Alternative cell lines for salmon alphavirus -1 isolation

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Salmonid alphaviruses have become an economically important threat to salmonid aquaculture in Europe. Salmonid alphavirus -1 (SAV-1), one of the 3 subtypes so far characterised, affects Atlantic salmon (*Salmo salar*) causing mortalities and growth retardation. This virus was first isolated from Chinook salmon embryo -214 (CHSE-214) cells in 1995 in Ireland. Several cell lines have since been tested to grow the virus, although their actual virus isolation has not being discussed on those cell lines. In the present study we have evaluated Chum salmon heart -1 (CHH-1), CHSE-214 and Salmon head kidney -1 (SHK-1) cells lines for the isolation of SAV-1. Atlantic salmon were experimentally injected with an Irish isolate of SAV-1 (F93-125). Kidney samples were evaluated by cell culture and RT-PCR on Days 1, 3, 5, 7, 10, 14, 21, 42 and 90 post injection (p.i.). All Day 3 p.i samples (n=5) were RT-PCR positive and gave cytopathic effect (CPE) on CHSE-214 cells and therefore used in the study. The Day 3 p.i kidney homogenates were inoculated onto the cell lines tested and examined daily for the development of a CPE. The CHH-1 cells produced a CPE from Day 6 p.i, while the CHSE-214 cells showed the presence of a CPE from Day 10. The CPE appeared as localised cell-rounding on both cell lines. In comparison, cells sloughing-off from the monolayer were noted in SHK-1 cells from Day 20 p.i. The virus was successfully isolated in subsequent passages on all cell lines tested indicating that all of these cells can be applied for the isolation and culture of SAV. The CHH-1 cell line, however has proven the most useful for diagnostic purposes, since the CPE developed the quickest in this cell line and it is now routinely used for diagnostics at IoA.

O-10
Infectious salmon anaemia virus HPR0 surveillance in marine Atlantic

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Infectious salmon anaemia virus (ISAV) is a serious and commercially important pathogen of Atlantic salmon. The virus has multiple types based on a highly polymorphic region (HPR) of the haemagglutinin-esterase (HE) gene located on genomic segment 6. A variant with a longer HPR type, HPR0, was first detected in Scotland in 2002 by PCR and sequencing and

has since been reported from several countries. The pathogenic viruses causing disease outbreaks all have a deletion in this region. The aim of this study was to assess the potential distribution of ISAV HPR0 across Scotland to help understand the risk posed by it's presence if found. Anonymous samples of gill and heart tissues from marine Atlantic salmon farms throughout Scotland were collected and screened for the presence of ISAV using a sensitive real-time PCR method. DNA sequencing was carried out on the positive samples to determine their HPR type. ISAV was detected in several samples originating from different locations. Sequence analysis indicated the virus was of the HPR0 type.

O-11
Antibody response of the Atlantic salmon (*Salmo salar* L.) against a soluble form of the ISAV hemagglutininesterase

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The efficiency of a vaccine is determined both by the immunogenicity of the antigen and whether or not a response is mounted against a relevant epitope. The hemagglutinin-esterase (HE) is one of the two major surface proteins of the infectious salmon anemia virus (ISAV). The protein is directly involved in the infection process by binding the virus to the surface of a host cell. The HE also displays esterase activity that is important for the release of viral progeny. Both of these functions are possible targets for neutralising antibodies. We have succeeded in the production and purification of a soluble form of the ISAV HE using insect cells. The recombinant HE has maintained both the hemagglutinating and esterase activity of its viral counterpart, which indicates that it is correctly folded and holds important conformational epitopes. The aim of the present study was to determine the usefulness of the recombinant soluble HE as an antigen, to map the location of major antigenic determinants, and to analyse the function of the induced antibodies. The results show that an antibody response is induced by the recombinant HE after i.p. injection in salmon, but that the response is poor compared to the response against a positive control antigen: *Limulus polyphemus* hemocyanin. Mapping of the epitopes and analysis whether or not the antibodies are neutralising are ongoing. In addition, efforts are being made to enhance the immunogenisity of the HE.

O-12
Gene expression and viral infection studies of Atlantic cod cells

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Atlantic cod (*Gadus morhua* L.) has received increased attention during recent years as a candidate for aquaculture. Knowledge of the immune system and which pathogens that represent a threat to a sustainable Atlantic cod farming industry is of major importance. During the past decades it has become clear that innate immunity plays an essential role in repelling an infection and also in directing the adaptive immune response. An important

research tool to study innate immune responses and host-pathogen interactions is suitable in vitro cell cultures. Our research group is currently studying cultured larvae cells and primary head kidney cells from Atlantic cod. Initial results of the suitability of these cells in innate immune response studies have shown that they express cytokines in response to immune stimuli. Cytokines like Interferon stimulated gene 15 (ISG15) and Interleukin-1β increase their gene expression in response to the dsRNA viral mimic, poly I:C. Also LGP2 which is known from mammals to function as an inhibitor of viral detection respond to poly I:C with elevated gene expression. Pilot studies have in addition shown that the cod larvae cells are susceptible to infectious pancreatic necrosis virus (IPNV) infection. These results show that cod larvae cells could be vital research tools in studies of both innate immune responses and of viral infections in Atlantic cod.

SESSION 3. BACTERIAL DISEASES OF FISH AND CAUSATIVE AGENTS

O-13
Epidemiology of *Renibacterium salmoninarum* in farmed salmonids in Iceland

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Renibacterium salmoninarum (Rs), the causative agent of bacterial kidney disease (BKD), is endemic in wild salmonid populations in Iceland. It is transmittable horizontally between individuals in sea and freshwater and vertically to new generations, i.e. inside eggs. All salmonid species are susceptible; Atlantic salmon is considered more susceptible than arctic char, which is more susceptible than brown trout and rainbow trout. Since 2003, BKD has caused increasing problems to Icelandic aquaculture, both in fresh- and seawater. Infection has been detected in 18 of approximately 40 farms presently in operation in Iceland. The source and progress of infection varies, in some cases the infection route seems clear but in other it is uncertain. Six to ten farms have for instance been traced to the same source. Sometimes clinical signs emerged shortly after first diagnosis but in other cases up to 3 years later. In yet other cases, clinical signs were not observed although ELISA tests were Rs-positive during regular monitoring for three years. During the last 5 years, millions of farmed salmonids of all sizes have been destroyed causing high financial loss in Icelandic aquaculture.

O-14
Renibacterium salmoninarum: a novel PCR and isolation of DNA from samples

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Screening for *Renibacterium salmoninarum*, the causative agent of BKD in salmonids is commonly carried out using an ELISA-test. For confirmation of such results, a different method is sometimes required. Nested PCR (nPCR) is one of the methods recommended by OIE. The main objectives of the study were to develop a safer and a more convenient PCR method and to find a simpler method for DNA isolation than using a DNA kit. In the ELISA-test chosen, polyclonal antibodies are used for antigen detection. In nPCR 4 primers that produce 2reactions are used. Template from the first reaction is transferred to a new tube, creating a considerable risk of contamination. A one-tube semi nested PCR (snPCR) was developed, involving construction of 3 primers that differ in annealing temperature and can therefore perform two reactions in the same tube. Bacterial culture, kidney tissue and ovarian fluid were subjected to various sample preparations. One of them involved dotting of samples on a special filter (FTA) where cell walls are broken down and nucleic acids preserved. A piece of the paper is put into the sample tube and works as a template for the PCR reaction. snPCR was shown to be cheaper, less time consuming and with lower risk of contamination than nPCR. Both tests have a similar sensitivity limit and detection capabilities comparable to the ELISA. Using FTA for DNA isolation is more convenient than using a DNA kit and the filter samples can be stored at room temperature for years.

O-15
Fish tuberculosis - a case study of host/pathogen interaction

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Fish tuberculosis is an infectious disease, which affects more than 200 species. *Mycobacterium marinum* is the main etiological agent of this disease. Fish are likely to be continuously exposed to mycobacteria in the environment, with clinical disease developing secondary to overcrowding, poor nutrition, deteriorating water quality, other stressful environmental conditions and advanced age. Special attention has been given to turbot (*Scophthalmus maximus*) tuberculosis because it is caused by a fish pathogen with a high prevalence rate and without an available vaccine. Thus, a study concerning the host/pathogen interaction has been done in order to a better understanding of the basic immunopathological reactions, contributing to the development of safer vaccine formulations to farmed turbot. In the present study, it has been demonstrated that early contact of macrophages with *M. marinum* induces respiratory burst with release of reactive oxygen intermediates, including

superoxide anion, from macrophages. Moreover, the present results clearly show that inactivated *M. marinum* elicited higher O2- production by macrophages compared with the response of the live pathogen. The different responses were also found in the cellular response. It is evident that live *M. marinum* is capable of inhibiting the release of superoxide anion to the extracellular space.

O-16
Experimental mycobacteriosis in Atlantic cod, *Gadus mo*rhua

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Farming of Atlantic cod is a fast expanding industry in Norway. Losses due to bacterial disease remain one of the main constraints in cod farming and the list of bacterial species pathogenic for cod is steadily growing. Recently, several outbreaks of mycobacteriosis have been identified in farmed Atlantic salmon in Norway. To establish whether mycobacteria pose a potential threat to cod farming, 170 cod (70 - 100g) were challenged with *Mycobacterium salmoniphilum*, recently isolated from farmed Atlantic salmon *Salmo salar*. The experiment included two groups (45 each group) of fish intraperitoneally injected with high and low doses of bacteria and a third larger (60 fish) cohabitant group. The experimental fish were maintained in a through-flow sea water system at a water temperature of 9 – 11°C. Twenty-four fish were sampled each time and examined macroscopically, histologically and bacteriologically at 2, 6, 14 and 22 weeks post infection (p.i). The findings of the study will be presented and the importance of mycobacteria as a potential pathogen of cod discussed.

O-17
Characteristics of rough and smooth colony types of *Flavobacterium psychrophilum*

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It is well known that certain fish pathogenic bacteria readily dissociates into different colony forms. This feature has also been reported for *Flavobacterium psychrophilum* although no further characterization has been done. After initial isolation of *F. psychrophilum* from diseased fish in the present study, two different colony types were observed. Upon repeated passages in broth culture, a change in growth mode of the bacterial cells occurred. A subsequent change in colony morphology was also observed. In order to evaluate the differences between these colony types, a detailed characterization was done. In total, four isolates of two different serotypes were included in the study. Growth mode, biochemical characteristics, cell surface hydrophobicity, adhesiveness and virulence of the cells of the colony types were examined. The composition of the cells was also characterized using SDS-PAGE. Two different colony types were observed in all isolates: A) smooth colonies with agglutinating cells and B) rough colonies with non-agglutinating cells. The cells of the smooth colonies showed higher hydrophobicity and adhesiveness, but the virulence of the two types

were different. No significant differences in biochemical reactions were observed between the cells of the two colony types. The significance of the two colony types is presently not known. The different characteristics of the two types might though be associated with survival and/or virulence strategies of the pathogen

O-18
Diversity with in the *Francisella philomiragia* group

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Infections with *Francisella* spp., francisellosis, are an emerging disease of farmed and wild fish and have been reported form a growing number of fish species on several continents. All reported *Francisella* spp. isolates from fish have ~99 % similarity in the 16s RNA gene to the human pathogen *F. philomiragia* subsp. *philomiragia*, but they constitute a separate clade on phylogenetic analysis of this gene. Within this clade are two genetic lineages of fish pathogenic *Francisella* spp. identified and they are further separated form the *F. philomiragia* subsp. *philomiragia* on basis of phenotypical tests and ability to give disease in mice. Nevertheless, little sequence data have been made available for these closely related bacteria. In present study have the diversity of six housekeeping gene loci of 35 isolates belonging to the *F. philomiragia* group been investigated by partial gene sequencing. These housekeeping genes demonstrate a grater sequence diversity than the 16s rRNA gene. Phylogenetic trees of individual genes are somewhat incongruent, but confirm the presence of at least three different species/subspecies with in the *F. philomiragia* group. However, the two *Francisella* species *F. piscicida* and *F. philomiragia* subsp. *noatunensis* are identical on all housekeeping genes investigated and constitute one species for which the epithet noatunensis have priority.

O-19
Challenge models for effluent mediated transmission of classical vibriosis and atypical furunculosis between fish species

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Waterborne transmission of fish pathogens between different fish species in aquaculture is an important issue. Particularly because there is increased interest in expanding the number of fish species in fish farming. In this study, challenge models for effluent transmission of classical vibriosis and atypical furunculosis between Atlantic salmon, cod and halibut have been performed by the use of a tank system designed for transmission by effluent. The system was tested with effluent transmission of classical vibriosis caused by *V. anguillarum* serotype O2a from infected salmon to cod. Subsequently infected cod had chronic infections with clinical signs such as petechial haemorrhages and ulcerations on the ventral abdominal wall, in addition to fin erosion. However, transmission of *V. anguillarum* serotype O2b could not be demonstrated from infected cod to salmon, as *V. anguillarum* could not be reisolated from

moribund or dead salmon. Transmission by effluent of atypical furunculosis from infected halibut to healthy cod and halibut was also confirmed. The mortality of cod and halibut in the receiving tanks was low, but physical stress seemed to provoke an outbreak in cod, but not in halibut. Transmission by effluent of francisellosis from infected cod to healthy cod is currently under testing and results will be presented at the conference.

O-20

Moritella viscosa subsp. *iridensis*, a "new" pathogen of farmed rainbow trout in Norway

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Winter-ulcer, caused by the bacterium *Moritella viscosa* is a serious disease of farmed salmonid fish, and represents one of the largest animal welfare challenges in Norwegian aquaculture. Although first recognised during the 1980's the bacterium was not named until 2000. Several serotypes are described from Iceland, but Norwegian isolates have been considered homogenous. Despite extensive vaccination, outbreaks of winter-ulcer continue to occur annually over large stretches of the Norwegian coastline. Against this background, a 3-year project involving Scannvacc AS and the Norwegian National Veterinary Institute, aimed at development of an improved vaccine was initiated. This project included investigation of strain homogeneity/ heterogeneity, to establish whether vaccine strains are representative of strains found in the field. Strains of *M. viscosa* were collected from various geographic areas and fish species and compared phenotypically and genetically. The investigated strains could be grouped both phenotypically and genetically into two main groups which display a high degree of host specificity for salmon and rainbow trout respectively. Given the consistent genotypical and phenotypical differences as well as host specificity, we consider that the isolates from rainbow trout merit subspecies status within *M. viscosa*. The publication process is now underway in which we propose the name *Moritella viscosa* subsp. *iridensis*. Iridensis (iridium = rainbow, ensis = belonging to)

O-21

Real-time PCR detection of the winter ulcer bacterium *Moritella viscosa* and immunogenic responses after bath challenge of Atlantic salmon

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Moritella viscosa is the main causative agent of winter ulcer, a disease that primarily affects salmonid fish in sea water during the cold water periods. The disease is initially characterised by localised swelling of skin surfaces followed by development of visible lesions. Gill pallor,

fin rot and severe internal pathology may also be observed. To gain more knowledge of the pathogenesis of *M. viscosa* in relation to winter ulcer, we performed a bath challenge of Atlantic salmon. 159 fish (80-110 grams) were exposed to challenge doses of 7x10^5 cfu/ml for one hour at 8-9 °C before continuous flow of sea water was introduced. The first mortalities were registered at day 2 post challenge. At day 13, 33 % cumulative mortality was reached. Blood samples for serology were taken and tissue samples of ulcer, muscle, head kidney, spleen, liver and gills were dissected for nucleic acid extraction. Bacteriological samples from tissue and blood were cultured on heart infusion agar supplemented with bovine blood and 2 % NaCl. *M. viscosa* was re-isolated from ulcer, head kidney, mucus, gills and blood, and serology showed low antibody titers. To detect bacterial DNA, a real-time PCR assay specifically targeting *M. viscosa* was developed. The number of genomic units of *M. viscosa* in tissue samples was quantified and a possible relation to expression of immune genes in fish known to respond to bacterial invasion was investigated.

O-22
Characterisation and virulence assay of an extracellular vibriolysin of *Moritella viscose*

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Moritella viscosa causes winter ulcer disease in salmonids reared at low temperatures. *M. viscosa* extracellular products are lethal to salmon and cause disease symptoms similar to those seen in fish infected with live bacteria. The aim of the study was to isolate and partially characterise an extracellular peptidase, termed MvP1, from *M. viscosa* and to evaluate its role in bacterial virulence. The isolated MvP1 was a 38 kDa metallopeptidase which degraded casein, gelatin and collagen. The optimum temperature for MvP1 was 40°C but the enzyme was active over a broad range of temperatures. MvP1 was nonlethal to salmon but caused severe hemorrhages and tissue necrosis at the injection site. MvP1 affected cell-cell adhesions in cultured cell lines but was not highly cytotoxic. The peptidase was non-hemolytic against erythrocytes but degraded IgM heavy chain. The gene encoding MvP1 was sequenced and encoded a 734 aa polypeptide containing signal sequence, a N-terminal propeptide, a catalytic domain and a C-terminal domain. The catalytic domain sequence showed highest similarity with vibriolysin (80% aa identity or lower). The consensus motif HEXXH-E was found within the catalytic domain. Several vibriolysins from pathogenic bacteria have been described as virulence factors, especially affecting invasion and colonisation. The results indicate that MvP1 is a previously unknown vibriolysin that may aid the invasion of *M. viscosa* into its host by causing tissue destruction.

SESSION 4. FISH PARASITOLOGY

O-23

Loma morhua infections in Atlantic cod (*Gadus morhua*): A molecular diagnostic assay for the elucidation of epidemiological factors during aquaculture

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Infection of Atlantic cod (*Gadus morhua*) with the protozoan parasite, *Loma morhua*, has impeded the full potential of the cod aquaculture industry in Atlantic Canada. The pathogen has adverse effects on both fish survival and their condition upon grow-out. Very little is known about routes of transmission or about other organisms that might contribute to the epidemiology of infections during aquaculture. The Internal Transcribed Spacer (ITS) of *L. morhua* ribosomal DNA (rDNA) was exploited to facilitate sensitive and species-specific diagnosis using PCR. Sequence alignments with related species, and assay using *L. salmonae* DNA, confirmed the specificity of this assay for *L. morhua*. Sensitivity of diagnosis was 100% using parasite material from 21 fish from two aquaculture cage sites. This contemporary diagnostic assay will aid in elucidating the life cycle and in studies on the epidemiology of infections. The sensitive and specific nature of this PCR assay also suggests excellent potential for diagnosis of *L. morhua* in live fish, but relies upon detection of circulating parasites during infection. Empirical assessment will determine the utility of PCR versus an antibody-based assay for reliable diagnosis of *L. morhua* infection in live cod. Elucidation of the life cycle and reliable diagnosis of infection are essential steps in the rational development of prophylactic measures to mitigate against disease outbreaks and for the assessment of efficacy of therapeutants.

O-24

Red vent syndrome (RVS) in wild Atlantic salmon in Scotland

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In Scotland wild Atlantic salmon, *Salmo salar* L represents a highly valuable resource largely through angling, associated tourism and commercial fishing. An estimated annual spend of over £73 million and 2200 full time jobs, mostly in remote rural areas are examples of their importance and governmental concern on their pathological conditions is reflected by the appointment of special commissions as early as in the 1930's. In June 2007 the Fisheries Research Service (FRS) was notified of returning salmon showing swollen, haemorrhagic vents and by September, over 50 rivers from all across Scotland reported similar findings as well as a number in England and Wales. Affected fish had only recently returned to spawn and externally, apart from the vents, they were in good general condition. Male and females were affected and the vast majority was grilse, with few two sea-winter fish recorded. FRS conducted a comprehensive diagnose screening of affected fish to establish the cause and

extent of the problem. Bacteriological, virological and molecular tests were negative for known fish pathogens, but histological and parasitological studies showed that all affected fish harboured very high numbers of nematode larvae in the discrete region of the vent. Histopathological changes included epidermal erosion, scale loss, haemorrhage, moderate-severe dermatitis and an inflammatory response mainly comprising eosinophilic granular cells (ECG's). Molecular analysis confirmed the identity as *Anisakis simplex* sensu stricto, based on RFLP patterns. The high number of larvae and the associated host reaction correlated with the swollen vents and considered to be its cause. There is no evidence that the condition prevented salmon from spawning in 2007; a prevalence survey will be pursued in the 2008 season.

O-25
Parasites of resident arctic charr, *Salvelinus alpinus* and brown trout, *Salmo trutta* in two lakes in Iceland.

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Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in two lakes in Iceland were examined for parasites. Altogether 40 fish, 10 of each species in each lake. At least 22 parasite species were found. Protozoa: *Hexamita salmonis*, *Apiosoma* sp. *Capriniana piscium* *Trichodina* sp., *Dermocystidium branchiale*. *Myxozoa*: *Chloromyxum truttae*, *Myxidium truttae*, *Myxobolus arcticus*, *M. cerebralis*, *M. neurobius*, *Sphaerospora truttae*. Helminths: *Apatemon gracilis*, *Diplostomum* sp., *Crepidostomum farionis*, *Phyllodistomum conostomum*, *Diphyllobothrium* sp., *Eubothrium crassum*, *E. salveli*ni, *Philonema onchorhyncii*, *Capillaria salvelini*. Crustacea: *Salmincola edwardsii* and *Sal*mincola (*salmonea*) sp. Six of the observed species are new records in Icelandic freshwater. In general, the total parasite fauna of the two lakes showed high degree of similarity, both with regard to micro- and macro-parasites. The majority of species were found in both lakes and many in/on both fish species. However, a considerable variability in prevalence and intensity between lakes and/or fish species was evident for certain parasite species. The results will be compared to other similar studies in Iceland as well as studies abroad.

O-26
Immunological parameters and parasite infection in chub *Leuciscus cephalus* in heavily polluted watershed

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The exposure to chemical pollutants and parasite infection may affect the physiological condition and immunological processes of fish host. In a field study, freshwater fish *Leuciscus*

cephalus was examined from 5 sites with different levels of chemical pollution in the Bilina River watershed, Czech Republic. Fulton's condition factor and hepatosomatic index did not differ among sites. Concentrations of PAHs measured from fish tissue showed no significant differences among sites compared to concentration in sediment. Parasite infection was significantly lower in polluted sites; parasite community in reference sites showed higher proportion of specialists and allogenic species. Immunological assays were performed to determine maximal intensity of respiratory burst and its total intensity, and lysozyme concentration as non-specific immune response parameters. Both respiratory burst activity and lysozyme concentration were moderately reduced in polluted sites. Due to the high variability in immunological parameters within each sampling site, no relationship with parasite infection was found. Livers, kidneys and gonads were histopathologically examined by HE-staining and analyzed for alterations by light microscopy. No significant alterations in any of the organs were observed. In conclusion, fish from polluted areas showed reduced parasite infection and immune response parameters, however no differences in fish condition or concentration of pollutants in fish were found.

O-27
Stress response in rainbow trout during infection with *Ichthyophthirius multifiliis* and formalin bath treatment

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Background: Ectoparasitic infections of fish are considered to be stress inducing. However, only a few studies have addressed this topic. In addition, formalin bath treatment of rainbow trout is a generally applied method of controlling ectoparasites, but the stress response in rainbow trout, *Oncorhynchus mykiss*, as a result of such treatments with formalin, has not received much attention and therefore needs further elucidation. Objectives: The present investigation addresses the stress-response induced by parasites and formalin treatment. Materials andmethods: Concentrations of plasma cortisol were monitored using ELISA. Samples were taken from groups subjected to confinement stress, infection with *Ichthyophthirius multifiliis* and formalin bath treatment. Results: Rainbow trout clearly responded to harmful stimuli by increasing plasma cortisol concentrations. Confinement, formalin bath treatment, as well as infection with *Ichthyophthirius multifiliis* resulted in significant high cortisol concentrations in plasma compared to the unhandled and uninfected control groups. Conclusion: The present study showed that cortisol release in rainbow trout is associated with infection with the skin ciliate *Ichthyophthirius multifiliis*. Formalin, which is used to control the parasite infection, also elicited a high production of this immuno-suppressing hormone in the host, indicating the possibility of secondary infections as a result of high dose formalin treatment.

O-28
X-cell disease (pseudobranchial tumours) in wild and farmed young cod, *Gadus morhua* L., in Iceland with a direct transmission study

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X-cells associated with tumours in pseudobranchs of cod are now recognized as being parasitic protozoans. The aim of the present studies was to determine the prevalence of these tumours in wild and farmed cod in Iceland and to make an attempt to demonstrate direct transmission of this parasite. Wild cod (4-22 months and 2-5 years) and farmed cod of wild and hatchery origin (0-2 years) were sampled and examined for the presence of x-cell tumours. In an infection trial disease free experimental cod were injected either orally or intraperitonially with tumour extract from naturally infected cod and examined two and four months after infection. Wild cod: The prevalence of x-cell disease was 7%, 3%, 6%, 23% and 7% in 6, 10, 18, 22 month old and 2-5 year old fish respectively. Farmed cod: Wild caught juvenile cod had natural x-cell infections (approx. 5% prevalence). During the first months of rearing many fish with tumours became emaciated. In the following months prevalence of tumours gradually declined, infected fish apparently had died. There were no signs of x-cell disease being transmitted from the fish of wild origin to disease free juvenile cod of hatchery origin when reared together. Infection trial: No x-cell tumours developed during the experimental period. The prevalence of x-cell disease seems to be greater in this study than formerly reported from cod. The infection trial did not support the hypothesis that x-cell parasites were capable of direct transmission.

SESSION 5. NORDFORSK SESSION: COMMUNICATION NETWORKS IN MARINE BACTERIA

O-29
The RNA chaperone Hfq regulates signal molecule production in *Vibrio anguillarum*

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In *Vibrio anguillarum*, VanM synthesizes N-acylhomoserine lactone signal molecules. The signals modulate the quorum sensing (QS) regulatory pathway inducing expression of small regulatory RNAs, which together with the RNA chaperone Hfq repress VanT expression. VanT is a central regulator of QS affecting biofilm, protease and pigment production. Using a vanM::gfp reporter gene fusion, vanM expression showed two peaks at early-log and early stationary phase suggesting that signal production has multiple regulatory controls. Hfq facilitates interactions with sRNA and target mRNA resulting in repression or activation of translation. Secondary mRNA structure analysis predicts an Hfq binding site upstream of the ribosomal binding site. In an hfq mutant, vanM expression was induced 10-fold compared to

the wild type. The vanM RNA half-life was shown to be 5-fold increased in the hfq mutant compared to wild type. Present work focuses on finding sRNAs that repress vanM expression. In summary, Hfq was shown to regulate vanM expression, suggesting that sRNAs are involved in vanM regulation. Regulation by sRNAs allows the bacteria to respond quickly and reversible to stress response and environmental signals, suggesting that QS may be regulated by environmental signals as well as population density

O-30
"Cytotoxic, adhesive and invasive properties of the fish pathogen *Moritella visc*osa"

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Moritella viscosa is the causative agent of `winter ulcer´, a disease that affects cultured salmonid fish in cold marine waters. The pathogenesis of this disease is poorly understood and only some virulence features have been characterized in *M. viscosa*. The pathological changes of `winter ulcer reveal extensive skin damage, indicating that the causative agent has the capacity to degrade host cells. To analyse and obtain a better understanding of *M. viscosa* cytotoxicity, we have utilized an infection-assay with a chinook salmon embryo cell line (CHSE). Cytotoxic, adhesive and invasive capabilities of *M. viscosa* have been studied using phase contrast microscopy and a double fluorescence-labelling technique that differentiates between intracellular and extracellular bacteria at different time points. Preliminary results show degradation of CHSE cells within few hours of infection. The study will clarify whether *M. viscosa* has the ability to invade host cells and may contribute to the understanding of pathogenesis of winter ulcer disease.

O-31
Generation and phenotypic screening of a *Vibrio salmonicida* transposon mutant library

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Vibrio salmonicida is the causative agent of cold-water vibriosis. The molecular mechanisms of host colonization, invasion, growth and virulence properties are still largely unknown. To be able to understand how the pathogen interacts within its own cell population by quorum sensing (QS) and with the fish host to establish a septicemic infection, a *V. salmonicida* random mutant library was generated. Genetic mutants have proven particularly useful when analyzing a pathogen's response to its host environment. Generation of the random mutant library was performed by using a high-efficiency Tn5-based erythromycin-resistant mini-transposon which was moved into the sequenced wild type *V. salmonicida* LFI1238 strain by conjugation. A number of phenotypic screens were performed to find mutants in the library with changes in properties related to virulence and the QS-system. A

motility assay was performed using semi-solid LB plates, measuring the motility zone of each mutant and comparing the results against LFI1238. To find mutants with changes in luminescence properties, each mutant's luminescence capacity was measured after adding caprinaldehyde ($C_{10}H_{20}O$). A modified biofilm assay was established using a minimal medium during incubation, followed by staining and elution of the biofilm using crystal violet and ethanol, respectively. The transposon insertion site was determined for strains yielding marked phenotypical changes by using arbitrary PCR and sequencing.

O-32
Whole genome sequencing of *Vibrio salmonicida* as platform to study basic mechanisms in virulence

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Vibrio salmonicida is a moderate halophilic ("salt loving") and psychrophilic ("cold loving") curved gram-negative bacterium with 3-9 polar flagella. It is the causative agent of cold-water vibriosis (Hitra disease), and infects different fish including salmon, cod and rainbow trout. However, the molecular mechanisms of host invasion, species specificity, colonization, growth and virulence properties are largely unknown. To provide a better understanding of the molecular nature of host-pathogen interactions between Atlantic salmon (host) and *V. salmonicida* (pathogen), we are currently characterizing basal mechanisms involved in virulence. These include oxidative stress, iron limitation, quorum sensing and motility.

An overview of main activities in our laboratory that include both experimental and computational work will be presented. For example, we have recently completed the sequencing and annotation of the *V. salmonicida* genome and found surprising features. A whole genome microarray was created based on the annotated genome, and we are currently investigating the transcriptome under various stress conditions. Finally, our goal is to apply powerful computer-based analyses to expand our insights into *V. salmonicida* virulence.

SESSION 6. FISH HEALTH AND PROPHYLAXIS

O-33
Improved vaccine against *Moritella viscosa*

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"Winter ulcer" is a disease in farmed fish. It is known from farmed Atlantic salmon and rainbow trout. The Gram negative bacterium *Moritella viscosa* is regarded the main cause of the disease. Despite comprehensive vaccination in a 10 year period, there have still been

problems with winter ulcer, and *Moritella viscosa* is being isolated from a large portion of the fish. *Moritella visco*sa isolates from 33 outbreaks of winter ulcer have been investigated regarding phenotypic and immunological variation. From Atlantic salmon the dominating strains show little variation. A modified vaccine has been tested in controlled trials and in the field. This vaccine is showing promising results with improved protection against *Moritella viscosa* and reduced side effects.

O-34
Combined effects of stress and vaccination on the metabolic rate of Atlantic salmon smolts

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The metabolic responses of salmon smolts to repeated stress and vaccination were examined using static respirometry and correlated with the response to exogenous dexamethasone and ACTH administration. Unvaccinated Atlantic salmon smolts were divided into three groups. Group 1 was subjected to a repeated stressor (lowering the water level in tank) daily for 5 wks. Group 2 were vaccinated with Alphaject 6-2 vaccine then subjected to the same stress regime as group 1. Group 3 acted as controls and was not handled except for sampling throughout the period of the study. Routine and maximum oxygen consumption rates were measured along with fish from each group being injected with dexamethasone (suppression test) and then 24h later ACTH (stimulation test) or PBS, and plasma cortisol levels thereafter determined 1h post-injection at the start of the stress regime and then again after 5 weeks. At the same time groups of initially, only the vaccinated group had an elevated routine metabolic rate, which remained elevated throughout the 5 wks of the stress period with a significantly reduced metabolic scope compared with the other groups. Both stressed groups showed an increased cortisol response to injection with ACTH compared to the control group. Only the vaccinated-stressed group showed failure in negative feedback system of the HPA axis when subjected to dexamethasone. This suggests a metabolic cost of vaccination but not stress and a major modification of the HPA axis.

O-35
Management of infectious salmon anaemia (ISA): Can vaccination prove to be an effective management tool?

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ISA is a viral disease that has caused major disease outbreaks in marine-farmed Atlantic salmon in the Northern Hemisphere and also in Chile since 2007. The causative agent, ISA virus, is an orthomyxovirus that resembles the influenza viruses and is classified as an “other significant disease” by the OIE. In Canada, left unchecked, an average mortality of 16.7% was experienced and varying levels of pathogenicity between strains can result in mortality more

than 50% in acute cases. Common control measures common to all geographical regions include use of single year class farms, surveillance, early detection and removal of infected fish, fallowing and bio-containment of blood water and processing waste. The local regulatory management of ISA has differed in the effected regions with varying results. Vaccination has been used concurrent to other management practices in North America and in the Faeroe Islands. The active development and testing of clinical relevance of ISAV vaccine since 1997 will be presented and discussed; including efficacy and potency test standardisation, validation of cohabitation challenge versus injection challenges, efficacy of Canadian ISA vaccine against genetically different strains, virus shed spread capacity from vaccinated fish, use survey studies, controlled studies in SW land based facilities, interference testing for use of molecular diagnostic test methods and vaccine use, and efficacy of ISA vaccines in multi-site clinical evaluations.

O-36
From laboratory to the field: Was the timing right to take an experimental AGD vaccine to sea?

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Amoebic gill disease (AGD) caused by *Neoparamoeba perura*ns is an ongoing problem for the Atlantic salmon industry in Tasmania. Although affected fish are safe to eat, treatment and associated lost productivity costs the industry in excess of Au$20million p.a. Currently freshwater bathing is used to treat AGD, however this is expensive, time consuming and not sustainable in the long term. Therefore alternate methods are required. CSIRO in conjunction with the University of Tasmania have been developing a six antigen DNA vaccine that affords approximately a 40% increase in protection in a controlled laboratory time to morbidity trial. However, the trigger for bathing fish in the production setting is the 'average' gill score for a cage. Therefore, to ascertain the commercial benefits of the experimental vaccine we PIT tagged and vaccinated 3000 fish. These fish were then sent to sea in the Spring of 2007 and stocked into separate cages either as 100% control, 100% vaccinated or a 50:50 mix of vaccinated and control fish with two replicates of each cage (6 cages in total). Fish were then subjected to normal production practices and routinely sampled to determine gill scores. When a cage reached a pre-determined threshold for bathing all fish were measured (gill score, weight and length) and the data collated. Each cage was measured 3 times over the production cycle. The results from the sea trial and their relevance to the laboratory results will be discussed in detail.

O-37
Th1 and Th17 responses after *Vibrio anguillarum* vaccination in the gadoid haddock (*Melanogrammus aeglefinus*)

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The classical division of T helper cells into two subsets, Th1 and Th2, has recently been challenged as four lineages of naïve CD4+ T cells have been recognised. These are Th1, Th2, Th17 and Treg, each of which produce a specific set of cytokines and the differential regulation of B-cell and T-cell responses. Interferon-gamma (IFN-g) and interleukin-22 (IL-22), hallmark cytokines from Th1 and Th17 cells respectively, were cloned in the gadoid haddock (*Melanogrammus aeglefinus*). A vaccination experiment in haddock designed to study the Th1 and Th17 responses was carried out. The vaccines consisted of two doses (high, low) of formalin-killed bacteria (*Vibrio anguillarum*, serotype O2) and a control group vaccinated with PBS. The high dose vaccine (HVang) contained 10^7cfu/ml, a dose known to elicit a good level of protection. The low dose (LVang), with a low count of bacteria (10^3cfu/ml), was intended to act as a low efficiency vaccine. Two months post vaccination fish were challenged by immersion in a bath containing 10^7cfu/ml *V. anguillarum* MT2582 for a period of 30 min. Compared to the control group, vaccinated fish had a relative percentage of survival (RPS) of 75% in the LVang group, while the fish from the HVang group had an RPS of 100%. Efficacy of vaccine preparations were then compared between groups in terms of immune gene expression analysed by real-time PCR, in samples both from different time-points post-immunisation and post-challenge.

O-38
Oligonucleotide microarray: An effective platform for antimicrobial resistance genotyping

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Numerous antimicrobial resistant pathogenic bacteria have emerged in aquaculture worldwide. To be able to do large-scale drug-resistance genotyping, we established an oligonucleotide microarray platform, consisted of 153 kinds of drug-resistance genes. Thirty four kinds of different Inc. group R-plasmids and various drug resistance fish pathogenic bacteria, which included *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *Lactococcus garvieae, Photobacterium damselae* subsp. *piscicida* and *Vibrio anguillarum* were assessed. The detection results of resistant bacterial phenotypes and genotypes of *Escherichia coli* carrying different Inc. group R plasmid showed significant consistency. The drug-resistance genes, such as chloramphenicol, kanamycin, streptomycin, sulfonamide and trimethoprim resistance genes were successfully detected and classified from cultured fish pathogenic bacteria. Moreover, bacterial drug-resistance genes were detected directly from *L.*

garvieae or *P. damselae* subsp. *Piscicida* infected yellowtail (*Seriola quinqueradiata*). In conclusion, the microarray platform may allow for rapid detection and classification of antimicrobial resistance of diverse bacteria.

O-39
A Saprolegnia parasitica cDNA library: Mining expressed sequence tags (ESTs) for potential vaccine candidates

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Saprolegnia parasitica is a fish pathogenic oomycete capable of causing disease in freshwater fish species. Since the global ban of the preferred control agent malachite green, *S. parasitica* infections have become a re-emerging problem for aquaculture, causing major losses worldwide. It is therefore essential to find a new and effective control strategy. Our work is aimed towards the development of a vaccine against *S. parasitica*. An infection model was established using Atlantic salmon *Salmo salar* and a cDNA library was constructed using *S. parasitica* mycelia interacting with the Atlantic salmon host. Sequencing of 3000 ESTs has given an insight into the genes transcribed at this crucial point in the *S. parasitica* lifecycle. Both *S. parasitica* and *S. salar* genes identified in the library are of interest to our research. To assess the *S. salar* response to a *S. parasitica* infection, quantitative RT-PCR has been performed on genes sequenced from the library and revealed numerous genes that are significantly up-regulated during infection. The *S. parasitica* genes sequenced from the library form the basis of the selection of vaccine candidates. By expressing these genes in salmonids, we hope to identify a protein from *Saprolegnia* that induces an immune response in the host. Ultimately, this work may lead to the development of a commercial vaccine that has the potential to alleviate the problems caused to the aquaculture industry due to *S. parasitica*.

O-40
Application of putative probionts at larval and juvenile stages of Atlantic cod (*Gadus morhua* L.) rearing

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It is anticipated that the use of probiotics may improve larval survival, growth and development during the first months of life. The aim of this work was to validate the use of putative probionts both at larval and juvenile stages of cod rearing through bathing and feeding treatments, respectively. In one rearing trial, a putative probiotic mixture of two bacterial strains was applied through bathing of ova at late post-fertilised stage and at larval stage with four replicates during the first four weeks of life. Microbiological analyses were performed and on day 36 post-hatch (ph), larval survival and growth assessed. Immunological

larval proteins were analysed by Western blots and enzymatic activity by casein and gelatin zymograms (4-14-21-28 dph). In another rearing trial, cod juveniles (10 ± 1g) were fed expanded dry feed for 27 or 54 days, either untreated (control) or treated with the probiotic bacteria, in mixture or separately. The results show that bacterial bathing at early larval stage led to significantly ($p<0.05$) higher larval weight, length and culturable microbial load in larval gastrointestinal (GI) tract. Protein analysis demonstrated that larval development occurred earlier in bacteria-treated larvae. Juveniles fed a certain probiotic dry feed grew significantly faster and had lower GI vibrio counts. The results suggest that these probionts affected the GI microflora and contributed to larval and juvenile digestion, either directly or indirectly.

O-41
Immune response in gilthead seabream *Sparus aurata* induced by a potential probiotic live yeast *Debaryomyces hansenii* CBS 8339

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Actually, the use of probiotics in aquaculture has been shown great interest principally on protection of infection diseases (Gatlin, 2002). The beneficial role of yeasts is being of particular interest because they represent an important source of beta-glucans, chitin, and nucleotides to increase disease resistance in fish (Ortuño et al., 2002). The purpose of this study was to evaluate the effects of dietary administration of the live yeast *Debaryomyces hansenii* CBS 8339 on immune responses in gilthead seabream (*Sparus aurata* L). Fish were fed during four weeks with a supplemented diet with marine yeast *D. hansenii* CBS 8339 equivalent to 10^6 $CFUg^{-1}$ and a control diet deprived of yeast. Humoral and cellular innate immune parameters and antioxidant enzymes were measured from serum, head-kidney leucocytes and liver, respectively, after 2 and 4 weeks of feeding. Expression levels of immune-associated genes, Hep, IgM, TCR-b, NCCRP-1, MHC-II alpha, CSF-1R, C3, TNF alpha and IL-1b, were also evaluated by real-time PCR in head-kidney, liver and intestine. *D. hansenii* administration in gilthead seabream significantly enhanced leucocyte peroxidase and respiratory burst activity at week 4. Phagocytic activity had significantly increased by week 2 of feeding yeast; cytotoxic activity was also higher at the same time. A significant increase in superoxide dismutase activity was observed at week 2 of feeding with the supplemented diet. Finally, the yeast supplemented diet up-regulated the expression of the nine genes in head-kidney. In liver and intestine only C3 was up-regulated. These results strongly support the idea that live yeast *D. hansenii* strain CBS 8339 can stimulate the innate immune parameters in seabream, promoting safe farming.

Poster presentations

1. FISH IMMUNOLOGY

P1-1
Toll-like receptors in Japanese flounder, *Paralichthys olivaceus*

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Toll-like receptors (TLRs) recognize specific pathogen-associated molecular patterns (PAMPs) to initiate immune responses. They were also reported orchestrating innate and acquired immune responses. Hence, understanding the TLR signaling in teleost fish could provide important information about the perceived uniqueness and complexity of teleost fish immunity due to genetic diversification. Moreover, knowledge about TLRs and their signal pathways will elucidate host-pathogen interaction that can be useful for the development of pathogen control methods such as vaccine adjuvants in aquaculture species. Here, we conducted identification of TLR genes in a commercially important marine fish species Japanese flounder, *Paralichthys olivaceus*. The Japanese flounder TLR genes were isolated using degenerate primers and from Expressed Sequence Tags (ESTs). We have succeeded in finding nine TLR homologs including TLR1, TLR2, TLR3, TLR5 (soluble form and membrane form), TLR7, TLR9, TLR21 and TLR22 from Japanese flounder. All the Japanese flounder TLRs contain functionally important structural features such as extracellular leucine rich repeats and intracellular Toll/interleukin receptor domain. Phylogenetic analysis of the Japanese flounder TLR genes showed that they group with their respective counterparts from other species. Most of the Japanese flounder TLR genes were expressed in organs involved in immune functions including kidney, spleen and peripheral blood leukocytes (PBLs).

P1-2
The seasonal changes in complement activity of common carp (*Cyprinus Carpio*)

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The fish non-specific immune response includes cellular (phagocytes) and humoral parts (complement system mainly) or the systemic inflammation. Complement system is composed of more than 30 proteins and three pathways of its activation have been recognised: classical, alternative and possible lectine. The total bacteriolytic activity (TA) including all pathways and activity of the alternative pathway (AP) were assessed in the plasma of common carp, *Cyprinus carpio* (Cyprinidae). The samples were collected in five periods of one year including different seasons and all together 80 males and 80 females were used.

Bioluminescence-based method of complement activity uses bioluminescent recombinant strain of E. coli; there is a correlation between light production and viability. The time needed for 50% killing of bacteria by plasma sample was used as the measured parameter. Plasma in concentration 300µl/ml needed approx. 1.5 hours in TA; AP showed lower bacteriolytic activity (approx. 1.7 hours) and moreover, needed higher plasma concentration (500 µl/ml). The seasonal changes in complement activity were observed, the highest TA was determined in autumn, lower in the summer. This activity in spring and winter was the lowest, nevertheless very similar each other. Concerning AP the highest activity was detected in autumn, lower in spring and the lowest values were determined in winter and in summer.
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P1-3
Functional and expression analysis of interleukin 2 (IL-2) in rainbow trout (*Oncorhynchus mykiss*)

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The control and prevention of infectious diseases is a major goal in fish farming. Therefore, knowledge of the fish immune system is of practical importance. Cytokines are single polypeptides or glycoproteins that act as signalling molecules within the immune system. This work is focused on the study of interleukin 2 (IL-2) in rainbow trout (*Oncorhynchus mykiss*). IL-2 is synthesized and secreted primarily by T-helper cells, and regulates T-cell responses, natural killer cells, B cells, monocytes/ macrophages and neutrophils. It may also have a potential role in protection against bacterial and viral diseases. Following our recent cloning of IL-2 in salmonids, analysis of factors affecting IL-2 expression was undertaken. Head kidney leucocytes were incubated for 4 h with different stimulants, then RNA was extracted, reverse transcribed and real-time PCR performed. The bioactivity of recombinant IL2 (rIL-2) was also studied. Following production in *E. coli* the rIL-2 was added to leucocyte cultures for 4 h at different concentrations and the effect upon immune gene expression studied. rIL-2 was found to increase its own expression, and the expression of STAT5, an important molecule involved in IL-2 signalling, with the largest effect seen using 1 ng/m The next experiments will study whether IL-2 can be used as a marker of specific cell-mediated immune responses in fish, and whether IL-2 has value as an adjuvant to increase fish vaccine efficacy.

P1-4
The acute phase response of cod (*Gadus morhua* L.)

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Acute phase response is an immunological and physiological response to injury, trauma or infection. So-called acute phase proteins have been shown to increase manifold in serum of mammals and some fish following acute phase induction. Of the acute phase proteins the pentraxins, C-reactive protein (CRP) and serum amyloid P (SAP), have been studied extensively. Two types of CRP-homologues, CRP-PI and CRP-PII have been demonstrated in cod. In this study the effects of induced acute phase response on the pentraxins and other immune parameters of cod were examined. Intramuscular injection of turpentine was used for the induction. Serum samples were collected at intervals for the analysis of various humoral parameters and liver samples were collected for the analysis of the gene expression of CRP-PI and CRP-PII using quantitative RT-PCR. In one experiment head kidney leukocytes were isolated at intervals from turpentine induced fish for respiratory burst analysis using Amplex Red Hydrogen Peroxidase Assay Kit. Results showed that turpentine induction resulted in reduced serum protein levels and an increase in cortisol. Turpentine had no measurable effect on the quantity or properties of CRP-PII in serum or on the expression of this protein in the liver. Turpentine induction reduced the respiratory burst activity of cod leukocytes.

P1-5
The natural antibodies of cod (*Gadus morhua* L.)

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Natural antibodies are present in the serum of vertebrates regardless of external antigenic stimulation. Characteristic activity is detected against haptenated proteins, single stranded DNA and thyroglobulin. Natural antibodies are believed to provide an instant protection against pathogens of a broad specificity and participate in homeostasis. Cod shows generally poor specific antibody response but shows a relatively high levels of natural antibodies and IgM in serum. In this project the specificity of natural antibodies was tested against several antigens using immunoblotting. Activity (titre), affinity and antigen driven selection of natural antibodies against TNP-BSA were examined using ELISA techniques and ammonium thiocyanate elution. The effects of environmental temperature, age, immunisation and infection on these parameters were studied. The results showed that natural antibodies of cod have characteristic but variable specificity for a panel of antigens, the activity being primarily directed against TNP-BSA, chitosan and polymannuronic acid. Increasing environmental temperature, increasing age and immune stimulation resulted in increasing activity of natural antibodies. Increasing activity was often commonly accompanied by decreasing affinity. In individuals that showed high specific and natural antibody activity the affinity of natural

antibodies was higher than that of the specific antibodies. Natural antibodies showed antigen driven antibody selection.

P1-6
Isolation and characterisation of two C-reactive protein homologues from cod (*Gadus morhua* L.)

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The pentraxins, serum amyloid P (SAP) and C-reactive protein (CRP) are pattern recognition proteins found in both invertebrates and vertebrates. Pentraxins are known to play a role in the acute phase response of both mammals and fish. Pentraxins are pentameric structures generally composed of non-covalently associated identical subunits with recognition and an effector face. In the present study the main aim was to characterise cod pentraxins. Cod pentraxins were isolated from serum by affinity chromatography using phosphorylcholine (PC) agarose followed by further purification by ion exchange chromatography. Two pentraxin-like proteins were isolated, referred to as CRP-PI and CRP-PII, both showing affinity for PC, the definitive characteristic of CRP. These proteins varied in their overall charge, pentameric and subunit molecular size, glycosylation and N-terminal amino acid sequences. The CRP-PI protein was homologous with the CRP-like pentraxin previously described in cod but showed marked sequence homology with SAP of different species. The CRP-PII protein was a new CRP homologue, which was characterized by substantial individual heterogeneity with regard to the number, size and relative density of subunits, glycosylation and pI values. Such individual heterogeneity was not observed for the CRP-PI protein. The results indicate a large amount of allelic variation of cod pentraxins and that the CRP-PII variants might be encoded at multiple loci in the cod genome.

P1-7
Ontogeny of innate defence genes in Atlantic cod

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Teleosts hatch at the embryonic stage of life and are thus exposed to pathogens before lymphoid organs have matured and adaptive immunity has developed. During this phase embryos and hatchlings depend on maternally transferred defence components and innate immune responses. The identification of increasing numbers of innate defence genes has provided the basis for the study of their temporal expression during embryonic development. In the present study the appearance of expressed Atlantic cod (*Gadus morhua* L.) innate defence genes was characterised in unfertilised eggs, embryos and hatchlings by using Real time PCR. Defence genes such as ISG15, IL-1β, IL-8, IL-10, IRF1, IFNγ, pentraxin and G-type lysozyme were expressed at a low level during embryonic development. All of these genes were expressed before hatching and the expression increased as the larvae grew in size.

Some of the immune genes were expressed before fertilisation indicating that mRNA of maternal origin might be a defence mechanism in Atlantic cod. Altogether, these results indicate that components of Atlantic cod innate immune system could be essential for survival during embryogenesis and larval development. These are stages during which eggs and larvae are continuously exposed to a wide variety of pathogens present in the aquatic environment.

P1-8
Detection and isolation of CD4+ leucocytes in rainbow trout (*Oncorhynchus mykiss*) using antiserum against trout CD4

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T Helper cells (or CD4+ T cells) are a group of T lymphocytes that carry a CD4 marker on their surface. They exercise helper functions by releasing cytokines following contact with foreign bodies. Recently CD4 has been sequenced and cloned in rainbow trout (*Oncorhynchus mykiss*) and this sequence was used in DNA immunization of mice to develop a panel of anti-CD4 monoclonal antibodies. These antibodies were screened by FACS, to determine to which sub-population of peripheral blood leucocytes (PBL) they bound, and promising antibodies were selected for further characterisation. cDNA from magnetically sorted CD4+ and CD4– PBL were analysed by PCR to verify the expression of CD4, CD8, and IgM, markers which are predominantly found on Helper T cells, Cytotoxic T cells, and B cells respectively. The results show one antibody in particular (anti-CD4(12)) is a promising candidate for further characterisation.

P1-9
Atlantic halibut, *Hippoglossus hippoglossus* CD4: Cloning and characterization

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It is of great importance to characterize and understand how fish develop immunological memory since the necessity for new and better vaccines is increasing. In higher vertebrates the immunological memory is developed through two specific cell types, B- and T- lymphocytes. T lymphocytes consists of two major sets; CD4 positive T helper (T_H) cell and CD8 positive cytotoxic T (T_C) cells. The expression of both CD8 and CD4 is critical for attaining cell mediated immune defence and T-cell development in the thymus. CD4 co-receptor binds to MHC class II upon the recognition of the antigen-T cell receptor interaction. The T_H cells secret cytokines to stimulate the expression, proliferation and differentiation of B cells thus attaining antibody responses, or leads to macrophage activation for the destruction of the intracellular bacteria. This study has been focused on identification and characterization of

CD4 co-receptor in Atlantic halibut. A real time PCR assay was established for quantification of CD4 mRNA expression in different organs, and as expected thymus exhibited highest expression. Expression profile of CD4 co-receptor can be studied over different time periods to understand its regulation upon stimulation of fish with vaccines, other mitogens or upon experimental infection.

P1-10
Cod cathelicidins: Sequencing and characterization of the genes

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With the culture of marine fish species increasing, interest in ways to combat infection in such species has grown. One such avenue is the search for novel antimicrobials. Antimicrobial peptides (AMPs) are host derived molecules with a significant role in innate immune responses and have been studied extensively in mammals. Cathelicidins are a family of AMPs which are manufactured as pre-pro-peptides, and stored in secondary granules of neutrophils in an inactive form. When infection occurs, the peptide is cleaved to release the active mature form that effects microbicidal activity. Within teleosts, cathelicidins have only been identified in salmonids to date. We have recently searched the fish EST and genome databases to see if these molecules are present in other fish species, and have found that gadoids also possess cathelicidins. In this study we have characterized the cathelicidin genes present in Atlantic cod (*Gadus morhua*). Using bioinformatics approaches, a number of expressed sequence tags with significant homology to cathelicidins were found. These were used to design primers to amplify these sequences by PCR, and the gene products were cloned and sequenced. Two cod cathelicidin genes were identified, that differed in the length of their active mature peptides. Studies are on-going to confirm if these two cathelicidin genes are inducible, with the potential to offer some protection against infection for the aquaculture industry if stocks are immunostimulated.

P1-11
Mucosal immunology and epithelial barrier function in response to long term hypoxia in the Atlantic salmon, *Salmo Salar*

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In mammals, chronic inflammation of intestinal epithelia is a well known condition called irritable bowel disease (IBD). A disturbed interaction between the endogenous microflora, other luminal antigens and the host immune system characterises IBD. The initiation is not fully understood but involves increased epithelial permeability caused i.e. by environmental factors like infection, ischemia, social and metabolic stress. The impaired barrier function leads to activation of the mucosal immune system with increased expression of cytokines i.e. IL-1β, IFNγ and TNFα. The cytokines further increases the intestinal permeability which

maintains the inflammation and creates a chronic condition. In fish, sub-optimal conditions (i.e. hypoxia) during intensive aquaculture frequently lead to observations of disturbed primary barriers, suggested to be caused by stress. In the present study plasma cortisol levels, intestinal permeability and mucosal cytokine expression were assessed after one month of periodic hypoxia. The intestinal permeability significantly increased by hypoxic conditions and a tendency towards increased plasma cortisol was seen. Expression of IL-1β; was up regulated while the inhibitor of NFκB was depressed. This suggests a NFκB activated IL-1β induced increase in intestinal epithelial permeability of salmon similar to the model recently suggested for mammals. This may suggest that long term stress can create chronic inflammation of the fish gastrointestinal tract.

P1-12
Immune gene expression in Atlantic salmon macrophages after *Piscirickettsia salmonis* infection

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Piscirickettsia salmonis is a facultative intracellular bacterium and causative agent of piscirickettsiosis. In Chilean salmon farming this disease is a main problem and mortality levels up to 90% have been reported. The disease has also been recorded in Norway, Canada, Ireland and Scotland. The development of an effective vaccine has not yet been accomplished and few reports exist on the host immune response after *P. salmonis* infection. We have used cell cultures of Atlantic salmon (*Salmo salar* L.) macrophages to gain more knowledge about the innate immune response after *P. salmonis* infections. Isolated macrophages from head kidney were infected with two *P. salmonis* isolates and cells were harvested at several time points (6, 12, 24, 48, 72, 96 and 120 h) after infection. Quantitative real time PCR was performed to study the expression profile of immune genes. The gene expression results showed that Interleukin (IL)-1beta; was up-regulated with the highest level 12 to 24 h after infection, while Interferon (IFN)-alpha; and inducible nitrogen oxide synthase (iNOS) was up-regulated with a peak 48 h after infection. Transforming growth factor (TGF)-beta; was slightly up-regulated at 12h only and otherwise down-regulated. These results indicate which innate immune genes are modulated by *P salmonis* and will contribute to the knowledge base needed for development of effective vaccines against this pathogen.

2. *VIRAL DISEASES OF FISH AND CAUSATIVE AGENTS*

P2-1
Apoptosis induced cell death in salmonid alphavirus infection

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Salmonid Alphaviruses are enveloped, RNA viruses, that cause sever contagious diseases in salmonid fish. The cellular mechanisms associated with pathogenesis of the disease have not yet been elucidated. Recently an *in vivo* study carried out in our laboratory found apoptotic cells in the head kidney and blood of fish artificially infected with the virus, suggesting programmed cell death. Here we report the results of an in-vitro experiment, from which ultra-structural changes associated with SAV infection in cell cultures, were observed. Irish isolate (F93-125) of salmonid alphavirus subtype-1 (SAV-1) was absorbed onto CHSE-214 cell (MOI=1), and harvested at 1h, 4h, 8h, 24h, and 48h post-infection (p.i.). Ultrathin sections were prepared for electron microscopic study. Progressive nuclear and cytoplasmic changes were evident in virus-infected cells in comparison to control cells over time. Chromatin marginalisation at 8h and 24h post inoculation (p.i) and chromatin condensation at 24h p.i. were evident. Formation of electron dense multiple micronuclei, with loss of cytoplamic characteristics, were evident at 48h p.i. which was indicative of progressive apoptosis in infected cells. Numerous electron dense membrane-bound cell fragments with loss to nuclear organisation were also observed. These observations are indicative of apoptosis in infected cells possibly leading to cell death in salmonid alphavirus infections, supporting the findings of our in vivo studies.

P2-2
Nodavirus: Transmission modes in farmed fish in Norway

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The fish nodaviruses (betanodaviruses) are causative agents of viral encephalopathy and retinopathy (VER), and are a widespread group of pathogens affecting a large number of fish species. In Norway nodavirus has caused disease in farming of turbot and Atlantic halibut, and outbreaks of disease from both Atlantic halibut and Atlantic cod were reported recently. Studies have been undertaken to elucidate at which stage nodavirus is released from fish, and at which stage the fish are susceptible to infection. Transmission modes by cohabitation between persistently infected fish and healthy fish has been examined. It is under investigation whether nodavirus is able to accumulate and persist in mussels. It would be interesting to study possible transmission through feed or healthy fish feeding on diseased dead fish. A vaccine strategy should be considered and focus on prophylaxis is essential. The results from this study can be of great help for the industry.

P2-3
Mx expression in common carp (*Cyprinus carpio* L.) in response to spring viraemia of carp virus (SVCV)

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Mx proteins are type I interferon-inducible GTPases which are involved in antiviral defence of vertebrates by interfering with virus replication. Expression of Mx genes was studied in common carp (*Cyprinus carpio* L.) after infection and re-infection of fish with spring viraemia of carp virus (SVCV) at 15°C. Fish were exposed to SVCV (CAPM V 539, strain Jaroslavicky 97) in concetration 103 TCID50/ml by immersion for 2 h. Eight weeks later survivors were exposed to the same viral dose. The tissue samples were collected in time points 0, 2days (d), 4d, 7d and 14d in both cases. Gene expression was determined by quantitative real-time PCR method and expressed relative to ß-actin. Mx level in common carp was detected in samples of head kidney, medium kidney, spleen, liver, gut, gill and muscle of six fish in one time point. At day 4 post first infection the expression of Mx1 gene were significantly elevated in all tissue samples. The highest expression of Mx1 gene was observed in liver, the lowest in muscle and gill. During secondary infection the minimal up-regulation of Mx1 gene was observed in all organs. No expression changes were observed for MxG gene. Supported by EU project FP6 007103 IMAQUANIM and Czechgrant MZE 0002716201

P2-4
Effects of acute stress and iridovirus infection on innate and cell mediated immunity of pallid and shovelnose Sturgeon

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Evaluating immunological responses of endangered pallid sturgeon (*Scaphirhynchus albus*) and shovelnose sturgeon (*Scaphirhynchus platorynchus*) during collection, artificial spawning, and hatchery rearing can provide important information on their health status. Cytochemical and functional characterization of blood leukocytes was used to evaluate the condition of sturgeon during acute crowding and handling stress; and health status during an iridovirus outbreak. Pallid sturgeon responded rapidly to stress with an increase in blood eosinophils, heterophils and a decrease in lymphocytes. Iridovirus infected shovelnose sturgeon were sampled for function assays; histopathology to determine virus severity; spleen and kidney for molecular analysis; and blood smears. Spleens from clinical and subclinical fish were used to construct a cDNA library to identify up-regulated immune-associated genes. Analysis of the cDNA library from infected fish yielded 1424 sequences, and 214 sequences (including redundancy) that were associated with immune function. Identification of these molecules and house keeping genes, and their correlation with clinical pathology and histopathology of the disease is essential for the development of rapid, molecular tools for future investigation of immune system-virus interactions. Rapid clinical assessment of blood cytology paired with

PCR based gene expression analysis can aid in health status determination of sturgeon and assist in conservation efforts.

3. BACTERIAL DISEASES OF FISH AND CAUSATIVE AGENTS

P3-1
Study the ExeD secretin of *Aeromonas*

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Type II secretion system (TIISS) is related to pathogenesis of various bacteria and virulence factors are secreted via this pathway. The only outer membrane protein of TIISS is a secretin called ExeD in species of *Aeromona*s. Secretins are found in two other secretion pathways and are conserved proteins. The exeD gene of *A. salmonicida* subsp. *achromogenes* (Asa) was isolated, sequenced, cloned and transformed into *Escherichia co*li. The ExeD was expressed from *E. co*li and the recombinant protein isolated. Antibodies against a 14 amino acid sequence of the ExeD were produced and native ExeD isolated. Antibacterial and opsonic activities of the antibodies were measured. Conservation of ExeD was estimated by sequencing exeD genes from 9 *Aeromona*s strains and *Haemophilus piscium* and the sequences compared to exeD sequences from two published *Aeromona*s genomes. The study revealed that the ExeD secretin is highly conserved amongst the genus *Aeromonas*. It also shows that antibodies against a small part of the protein do possess specific opsonic and antibacterial activities against Asa and *Yersenia ruckeri*. The results indicate that ExeD may be a promising component in fish vaccines with a broad activity. This study also demonstrated that *H. piscium* contains TIISS secretin that is identical to the ExeD of *A. salmonicida*. The results therefore support that *H. piscium* needs to be reclassified as *A. salmonicida*.

P3-2
Immune response in sole (*Solea senegalensis*) against *Photobacterium damselae* subsp. *piscicida* antigens

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Photobacterium damselae subsp. *piscicida* is affecting the development of *Solea senegalensis* culture. Sole immune response against the bacteria is poorly known; however some vaccine designs (made with inactivated cells) have had a relative effectiveness against the sickness. The administration of subcellular antigens as vaccine could increase the effectiveness of the immune response. The aim of this work is the study of the specific immune response of *S. senegalensis* inoculated with extracellular polymeric substances (ECPs) and inactivated whole cells of *Photobacterium damselae* subsp. *piscicid*a. Fish were

immunizated by intraperitoneal injection with 0.1 ml formalin inactivated ECPs and whole cells of *P. damselae* subsp. *piscicid*a and Freund incomplete adjuvant (FIA, 1:1). Two months later fish were boosted with the same antigens in FIA. Serum from fish were collected to test by ELISA, using different subcellular fractions of bacteria as antigens (ECPs, capsular antigen, outer membrane proteins, LPS, O antigen and formalized whole cells). The specific response of the fish serum was tested by ELISA using inactivated whole cells of several marine bacteria as antigens. All the *P. damselae* subsp. *piscicida* subunits and whole cells showed a high antibody level. Significant differences were fount between control and immunized fish, but differences between first immunization and booster were not fount.

P3-3
Characterisation of the mv-ag in different serotypes of *Moritella viscosa*

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Moritella viscosa are Gram negative psychrophilic bacteria that causes winter ulcer disease in fish farmed at temperatures below 10°C. The bacteria have been isolated from fish samples in Iceland, Norway, Scotland, Canada and Faroe Islands. A previous study has shown the importance of a 17/19 kD antigen which is named after its size in two strains of the bacterium, the typestrain (478/88) from Norway is 17kD and an Icelandic strain (K2) is 19kD (1). The main goal of this study is to isolate and identify the gene that codes for the 17/19 kD antigen of *M. viscosa* for further characterisation, classification of different serotypes and vaccine research. Using Triton X-114 facilitated phase partitioning it was possible to isolate the antigen. Amino acid sequence was obtained through MS/MS analysis and a following database search resulted in a match of outer membrane protein of a close relative *Moritella* sp. PE36. The gene sequence of the coding gene, mv-ag, was used to design primers. Using touchdown PCR the mv-ag was amplified in 10 strains of *M. viscosa* isolated from Iceland, Norway, Scotland and Canada. The amplified gene of 10 strains representing different serotypes was sequenced and the results were compared.

References

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P3-4
Visualisation the first stages of Senegalese sole infection by GFP-tagged *Vibrio harveyi*

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Senegalese sole (*Solea senegalensis*, Kaup 1858) is one of the most valuable cultured fish in southern Europe. Intensive rearing often results in breakdowns due to several opportunistic

bacteria like *Vibrio harveyi* that can readily survive and multiply in the environment causing vibriosis and producing skin lesions, haemorrhaging ulcers, systemic septicaemia and mortality. The aims of this study are to examine the first stages of *V. harveyi* infection on sole, the route of entry into fish and the sites at which invasion occurs. Therefore, plasmid pVSV102 carrying gfp gene was transferred into bacterial pathogen *V. harveyi* Lg16/00 by conjugation employing triparental mating to obtain *V. harveyi* showed gfp expression. *Solea senegalensis* were immersed in seawater containing the labeled GFP-*V. harvey* during 8 hour. Two days postinfection, the infection was visualised at the whole fish and single bacterium levels. Fluorescent GFP-*V. harveyi* cells were observed in skin (in fish scales), and in gastrointestinal tract and gills using confocal and fluorescent microscopy. Furthermore, the gastrointestinal tract was observed intact inside the whole fish using fluorescent estereomicroscopy so this method allows the visualisation of the development of infection in live fishes. Vibriosis caused by *V. harveyi* Lg16/00 can be transmitted by the waterborne and the portals of entry for *V. harveyi* into Senegalese sole are gastrointestinal tract, skin and gills.

P3-5
Tagging the fish pathogen *Vibrio harveyi* with GFP and RFP by biparental and triparental conjugation

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Vibrio harveyi is an invasive pathogen of fish. The virulence factors like proteases and the ability to attach to fish surface provide the mechanism for the adhesion and colonization of host. In studies of bacterial-host interactions during bacterial infection, green fluorescent protein (GFP) and red fluorescent protein (RFP) have been used as biomarker. Nevertheless, the main limitation is the difficult of bacterial transformation and plasmid retention if a selective medium and specific outgrowth conditions are not available. In this work, two methods for GFP- and one for RFP-tagging *V. harveyi* have been evaluated. Simple conjugation by biparental mating method was carried out by using as donor *E. coli* carrying plasmids pEVS104 and pKV111 (GFP), and *V. harveyi* Lg16/00 strain as the recipient; pKV111 has p15A replication origin from *E. coli*. GFP-tagging *V. harveyi* was isolated from mating mixture by selective outgrowth. This method provides GFP-*V. harveyi*, but pKV111 is not stable during bacterial growth in the absence of antibiotic selection and is poorly retained. A second conjugation methodology by triparental mating has been applied using the conjugative helper *E. coli* carrying pEVS104, and *E. coli* as donor with pVSV102 (GFP) or pVSV208 (RFP), based both on pES213 from *V. fisheri*, which is more stable in absence of antibiotic and well retained by GFP -*V. harveyi* and RFP -*V. harveyi*. These strains can now be used in studies of bacterial-host interactions during bacterial infection.

P3-6
Mycobacterium salmoniphilum infection in farmed Norwegian Atlantic salmon (*Salmo salar* L)

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Mycobacteriosis (piscine tuberculosis) has been reported to affect a wide range of freshwater and marine fish species, however, reports from farmed salmon are rare, and the disease has not previously been described in Norwegian farmed salmon. During 2006 and 2007, a rapidly growing *Mycobacterium* sp. subsequently identified as *Mycobacterium salmoniphilum*, was isolated on two occasions from newly dead and moribund fish during outbreaks of disease in farmed Atlantic salmon (*Salmo salar* L) on the western coast of Norway. *M. salmoniphilum* was isolated (Middelbrook) in large numbers from fish with and without typical macroscopic lesions which included multiple greyish-white granuloma-like nodules in liver, kidney and spleen. Splenomegaly and ascites were additional findings in some fish. Histopathology revealed the presence Gram positive and Ziehl-Neelsen positive, long slender rods in circular densely packed nodules in the liver and spleen. Aggragates of acidfast bacteria were seen in association with pancreatic tissue and in the inflamed spleen capsule. Steatitis with fibroblast-like cells, leukocytes and clear spaces in the pyloric region were also observed. To our knowledge this is the first description of mycobacteriosis as a contributing factor to increased chronic mortality in Atlantic salmon in the Norwegian marine environment.

P3-7
Comparative susceptibility of turbot *Scophthalmus maximus*, halibut *Hippoglossus hippoglossus*, and cod *Gadus morhua* yolk sac larvae challenged with different serotypes of *Vibrio anguillarum* and *Vibrio* spp.

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In intensive aquaculture systems high mortalities are frequently observed during the early life stages of marine fish. The aim of this study was to investigate differences in susceptibility to various serotypes of *Vibrio anguillarum* O1, O2*α* and O2β, *Vibrio salmonidida* and *Vibrio splendidus* for turbot *Scophthalmus maximus*, halibut *Hippoglossus hippoglossus* and cod *Gahus morhua*. A multidish system was used, with one egg distributed to each well added 2 ml of sterile seawater and bacterial cultures. Final concentrations in the wells were 10^6 and 10^4 CFU ml^{-1}, respectively. Unchallenged eggs and larvae were used as controls. Larvae in challenged groups suffering from high mortality were examined by immunohistochemistry, using absorbed polyclonal antisera. The O2α serotype was highly pathogenic to all three species. The O1 serotype was pathogenic to halibut and cod. The immunohistochemical examinations revealed differences in pathology. The O1 serotype caused a more severe and developed pathology compared to O2α. In larvae exposed to O1 pathology and bacterial cells were seen in dermis, gastrointestinal tract, brain and eye area while in larvae exposed to the O2α serotype pathology was scarce and limited to the gastrointestinal tract. These results

could imply that there are differences in the immunity among the species or that the bacteria are host specific. The O2β did not cause a significant increase in mortality.

4. FISH PARASITOLOGY

P4-1
Trichinelloid nematode in muscle cavities in the swordfish, *Xiphias gladius*, from the Mediterranean Sea

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In a swordfish, *Xiphias gladius*, from the eastern Mediterranean, large tissue lumps were found within cavities in the neck region. Digital photos and ethanol-fixed tissue samples were studied for diagnostic purposes. Cavity cross sections were ~12 x 20 mm wide and carried numerous small clear eggs, ~15 x 20 µm, inside. Cleared tissue lumps contained denser internal structures with coiled strands and many eggs. Thin (2 µm), stained (toluidine blue) sections of tissue samples were studied by microscopy. They contained unpigmented oval eggs in tight groups. Between these groups, strings and rounded blue-stained "islands" were seen, some with eggs in them. The "blue islands" were identified as female nematodes within degenerate host tissue, voiding their eggs *in situ*. The nematode *Huffmanela paranoi* Moravec & Garibaldi, 2000 was described on the basis of dark brown ova in the epidermis of *Xiphias gladius* from the western Mediterranean Sea, but adult specimens were not found. Several fish parasitic trichinelloid species are known and named on the basis of their ova and hosts. However, the adults of only a few species have been described. Thus, with the present findings it seems likely that the actual parasite species is *Huffmanela paranoi*.

P4-2
Occurrence of *Kudoa thyrsites* (Myxosporea) in Atlantic mackerel from the North Sea

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Members of the myxosporean genus *Kudoa* occur in many marine teleosts worldwide. Several species are of concern to the fishery and aquaculture industries as they may induce post mortem myoliquefaction of the fish muscle, commonly referred to as 'soft flesh'.

In this study, the occurrence and host effect of a *Kudoa* species in Atlantic mackerel (*Scomber scombrus*) from the North Sea are described. During 2003-2007, numerous Atlantic mackerel were caught in the northern North Sea and examined for the occurrence of post mortem myoliquefaction and/or the presence of myxospores or extrasporogonic parasite

stages. Generalised myoliquefaction associated with *Kudoa* sp. occurred in 0.8% of texture tested fish (n=1339). There was a significant difference in the prevalence of myoliquefaction between medium sized (400-600g) and larger mackerel (>600g). The prevalence reached ~9% in the latter host size group. Preliminary *Kudoa* sp. specific PCR-testing of 70 mackerel indicates that the prevalence of extrasporogonic stages is high, reaching 90% in various organs of smaller fish (<400g). No inflammatory host response was associated with the presence of plasmodia within single host muscle fibres. Based mainly on analysis of the nuclear small subunit (SSU) ribosomal DNA, the present *Kudoa* species is assigned to *K. thyrsites*. However, due to the species' apparently wide geographical distribution and host range, along with the still unknown life cycle of *Kudoa* spp., the taxonomic status of *K. thyrsites* appears not to be fully resolved.

P4-3
Application of a real-time PCR assay to detect *Lepeophtheirus salmonis* in plankton samples collected in close proximity to Atlantic salmon sentinel

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Sea lice are marine ectoparasitic copepods belonging to the family Caligidae. They are important pathogens of the aquaculture industry in Scotland and abroad and have the potential to inflict extensive damage to their host. Of particular importance, is the 'salmon louse', *Lepeophtheirus salmonis*, which causes significant financial losses in Atlantic salmon aquaculture. The aim of this study was to utilise and assess the usefulness of a previously developed real-time PCR assay for detecting *L. salmonis* in plankton samples in an environmental situation. In addition, results could be analysed alongside lice numbers counted on sentinel cage fish as part of a related study. Plankton collectors were placed in close proximity to three sentinel cages each containing 50 Atlantic salmon at different sites in Loch Sheildaig on Scotland's West coast. Plankton samplers were run for up to 12 hours. Total DNA was extracted from plankton samples and analysed by real-time PCR targeting the *L. salmonis* COI gene. Samples were collected and analysed from April 2006-2008. Results indicated the presence of *L. salmonis* within plankton however preliminary analysis suggested little correlation between real-time PCR results and lice counts recorded off sentinel fish.

P4-4
The infestation with *Triaenophorus* spp. in rearing fish larvae by feeding zooplancton and strategies for escaping the problem

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During a case study in 2007 at the fish rearing station Kreuzstein in Scharfling on lake Mondsee (Upper-Austria) the appearance of procercoids of *Triaenophorus* spp. in zooplancton was surveyed in relation to season and species composition of zooplancton population in the lake. The objective was to establish strategies to avoid plerocercoid infestations in juvenile fish fed with zooplancton during the nursery period. Procercoids of *Triaenophorus* spp. were found between 23.5. and 3.7.2007 about 4-5 weeks after the start of pike spawning. Procercoids were detected by aniline staining. The mean invasion rate in *Cyclops* spp. at this time was 9,4 ± 12%, the maximum 38,7%. In *Diaptomus s*pp. procercoids were found only sporadic, in *Daphni*a spp. never. In feeding experiments the invaded living zooplancton represented a source of infestation with *Triaenophorus crassus* and *Triaenophorus nodulosus* for all tested fish larvae (*Thymallus thymallus* and *Coregonus* spp.). The infestation rates of *T. crassus* varied from 2%-5% with frequent occurrence in the first half of June, those of *T. nodulosus* 2%-9% with increased distribution in the second half of June. With respect to the seasonal defined procercoid infestation rates the feeding of susceptible fish species with living zooplancton should not be carried out in the critical period. Alternatives for this defined time are either feeding with deep frozen zooplankton, or – where possible – with artificial dry food.

P4-5
Molecular studies of the *Saprolegnia*-fish interaction

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The pathogenic-oomycete, *Saprolegnia parasitica* infects freshwater fish and is a particular problem in the aquaculture industry where it is estimated that 10% of all hatched salmon succumb to *Saprolegni*a-infection. In addition, *Saprolegnia* species have been associated with the decline of wild salmon populations around the world. The impact of the disease to aquaculture was previously minimized by the use of an organic dye, malachite green. However, the use of this compound has been banned resulting in a dramatic recrudescence of *Saprolegnia* infection, and as a result has increased the need to study and understand this important host-pathogen interaction. To enable us to study the fish *Saprolegni*a interaction we have developed an in vitro infection model, where a cultured-monolayer of a primary fish cell-line (RTG-2) is infected with cysts of *S. parasitica*. This model has enabled us to harvested material from several stages of the interaction between fish and *Saprolegnia*, allowing us to investigate the kinetics of the infection using a range of molecular, microscopic and biochemical techniques. This integrated approach has allowed us to begin addressing the

molecular mechanisms, which enable *Saprolegnia* to successfully infect fish, the processes that suppress host defences during infection, and the nature of the pathogen/host interaction. Our latest findings will be presented.

P4-6
Fish invasion disease in the pond carp farms of Ararat Valley, Armenia

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Objective: The revelation of invasion diseases of fish of Ararat Valley pond carp farms. Material and methods: The long-term collections of the helminths of 4 fish species from 4 carp farms of Ararat Valley serve as material. Total 1540 specimens of Cyprinidae have been investigated. Treatment of the helminths will be carried out by common methods. Results: The research has shown rather high fish invasion by helminths – 68%. 10 species of the helminths have been found: Monogenea - *Eudiplozoon nipponicum* Goto, 1891, *Dactylogyrus vastator* Nibelin, 1924, *Dactylogyrus extensus* Muller et van Cleave, 1932; Trematoda - *Diplostomum rutili* Razmashkin, 1969, *D. spathaceum* (Rudolphi, 1819), *D. paraspathaceum* Shigin, 1965 and *D. mergi* Dubois, 1932; Cestoda - *Bothriocephalus acheilognathi* Yeh, 1955, *Ligula intestinalis* (Linnaeus, 1758) and *Caryophyllaeus fimbriceps* Annenkova-Chlopina, 1919. They are parasites in the body, intestine, the gills and the crystalline lens of fish. Conclusion: The main helminthoses of 4 fish species of Cyprinidae from the carp farms of Ararat Valley have been revealed. These are diplostomosis, ligulosis, bothriocephalosis and dactylogirosis.

P4-7
Red vent syndrome in wild Atlantic salmon (*Salmo salar*) in Icelandic waters

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During the summer of 2007 an unusually high frequency of Atlantic salmon (*Salmo salar*) with red vent was noticed in Icelandic rivers. A total number of 38 wild Atlantic salmon (10 males and 28 females), migrating from sea, with a red vent syndrome were brought to our laboratory for examination. The extent of the lesion varied from being only a slight reddening of the genital papilla to a more widespread and extensive haemorrhage and necrosis covering the vent. Bleeding from the vent was a common observation. Nematodes were seen in or on various internal organs without significant pathological changes. The vent itself was also infested by nematodes, especially the spongy part and also the surrounding skeletal muscular tissue. Multifocal parasitic granulomas, haemorrhages and necrosis were frequently observed in the vent and surrounding tissue. No bacterial infection was detected, but viral examination was not carried out. Histological preparations, however, demonstrate clearly the significant involvement and tissue damage caused by the nematode larvae. It is our opinion that a nematode (in our case *Anisakis simplex* larvae) infestation is the reason for the red vent syndrome.

P4-8
Ultrastructure of the digestive system of *Macrogyrodactylus congolensis* Prudhoe, 1957, a mongenean skin parasite of the Nile catfish *Clarias gariepinus*

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Gyrodactylid monogeneans are potential pathogens of freshwater and marine fishes. *Macrogyrodactylus congolensis* live on the skin and fins of *Clarias gariepinus*. The aim of the present study was to illustrate the ultrastructure of the digestive system of *M. congolensis*. The pharynx consists of an anterior muscular and a posterior mainly glandular regions. The anterior region possesses 6 pharyngeal papillae while the posterior region has glandular syncytium and radial muscle fibres. The glandular syncytium possesses spherical, electron – dense granules. Oesophageal glands contain abundant, small, electron-dense secretory bodies. The intestine consists of a short median tube and two long, blind caeca. Both the tube and caeca have an uninterrupted syncytial gastrodermis resting on a fibrous basal matrix and possesses many lamellae, different kinds of vacuoles (V1, V2 and V3), many tubular structures, lipid-like droplets and melanin-like particles.The possible functions of these structures were discussed.

P4-9
Fecundity rate of *Caligus rogercresseyi* under controlled conditions

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Caligus rogercresseyi is one of the most serious pathological problems for the salmon farming in Chile. In order to get information about the fecundity rate of this sea louse, gravid females were collected from Atlantic salmon (*Salmo salar*) from a farm located in the area of Puerto Montt (41º46'S72º56'W). Eggs strings were incubated under controlled conditions to obtain virgin adult females. Females copulated by once time produced eleven generations of eggs strings in a period of 80 day. The first egg strings were produced after 320ºD since the egg incubation. The next generations of eggs strings were produced with a periodicity between 5 to 6 days at 12ºC. The egg size was keeping constant through the different generations of eggs strings, with a similar size recorded in the field (0.34 mm). The average length of the egg string (3.0 mm) and the number of eggs per string (30) was keeping constant until the generation eight. However, it showed a reduction in the next egg strings generations. Both, the values recorded in captivity for the egg string length such as the number of egg per string were minor than the values recorded in the field in previous studies. This research was carried out in the framework of the project Fonfef D04I12555 supported by Conicyt.

5. NORDFORSK SESSION: COMMUNICATION NETWORKS IN MARINE BACTERIA

P5-1
Type VI secretion and its putative role in *Vibrio anguillarum* virulence

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Vibrio anguillarum causes vibriosis in both, farmed and wild fish and has large impact on aquaculture. Recently, a new secretion system to transport proteins across the cell wall was discovered in many bacteria. This system is named Type VI secretion system (T6SS) and important for Vibrio cholerae virulence. One protein secreted by this system is Hcp (hemolysin coregulated protein). *V. anguillarum* contains a T6SS, which harbors a designated vasoperon (genes vasA–L) and an operon upstream with hcp, an Hcp protein different from *V. cholerae* Hcp. Western analyses of cell pellets and supernatants show that Hcp is exported into the extracellular environment. Deletions in components of the T6SS secretion machinery result in low to no detectable Hcp in the supernatant. Some T6SS mutants have altered VanT protein levels. VanT, the main regulator protein in the vibrio-specific quorum-sensing system of *V. anguillarum* is important for survival, production of extracellular proteases and biofilm formation, which are crucial for bacterial survival on the fish and virulence. LD50 data suggest that T6SS mutants are more effectively killing the fish. To gain more insight into the infection, *In vivo* Imaging and fluorescent microscopy is used. Preliminary data from fish infected via immersion show that T6SS mutants are faster in colonizing the fish and spread more on the surface. Deleting components of T6SS might deregulate the secretion system and lead to a less controlled virulence.

6. FISH HEALTH AND PROPHYLAXIS

P6-1
Using reverse vaccinology to design more efficacious antigens for vaccination against amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*)

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Amoebic gill disease (AGD) is particularly problematic for the Australian Atlantic salmon industry. So far traditional approaches to vaccine development for AGD have not worked. However, CSIRO currently has a 6 antigen DNA vaccine under development that affords an approximate increase in protection to mortality of 40% (please see presentation by M. Cook). Despite this there is still a need to identify further antigenic candidates that may lead to a more

efficacious vaccine. In recent times the advent of genomic technologies, combined with the principles of reverse vaccination, has led to a paradigm shift in the way vaccines are developed. In essence, this involves using genetic information (DNA sequencing combined with bioinformatic analysis) to predict and design potential vaccines *in silico*. This has been made possible through modern high throughput genomics capabilities and large public domain genetic databases. The reverse vaccine approach, through sequencing of Neoparamoeba genes and comparison with known antigens from other pathogens provides a very powerful way to identify potential AGD vaccine antigens. To this end we produced a normalised cDNA library from infective amoebae. Approximately 3000 resultant clones from this library were sequenced and subjected to bio-informatic analysis. The results of this work as well as the future directions for AGD vaccine antigen discovery and testing will be presented

P6-2
Vaccination of Atlantic salmon carrying IPNV of different virulence

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Infectious pancreatic necrosis (IPN) is an important viral disease in aquaculture causing substantial economical losses, especially due to mortality in Atlantic salmon post-smolts. The problem continues despite the use of IPN vaccines proven efficient in challenge trials. It is therefore important to gain more knowledge about the common asymptomatic carrier state of IPN virus (IPNV) in Atlantic salmon, and the risk of recurrence of IPN after transfer to seawater. This represents virus-host interactions of high complexity. The present study focuses on the impact vaccination of salmon persistently infected with IPNV of high or low virulence, may have on the protection against IPN. IPNV carriers were experimentally established by bath challenge early in the fresh water phase, vaccinated with or without an IPNV component, smoltificated and transferred to seawater. Non-carriers and saline-injected control groups were included. In addition, groups of carriers and non-carriers were challenged after seawater transfer. The experimental period was 5 months and mortality due to IPN in carriers or non-carriers, and in the differently vaccinated groups was registered. The results demonstrated that non-vaccinated carriers of IPNV represent a serious risk of IPN outbreak after smoltification and seawater transfer. Both high and low virulent IPNV may establish a carrier state, but the outcome after an IPN recurrence differs. Vaccination protects IPNV carriers against recurrence of IPN in seawater. The effect was comparable to that observed in vaccinated non-carriers following IPNV challenge in seawater. The carrier state showed no negative impact on the protective effect of vaccination against a new IPNV challenge after seawater transfer.

P6-3
Probiotic bacteria in halibut larviculture

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Potential probiotic bacterial strains were isolated from samples of surface sterilized larvae from all production units of two spawning groups at a commercial halibut farm site. The strains were selected with respect to a relative dominance in the gut of larvae in tanks with an overall successful outcome. Of 384 bacterial isolates tested, 18 strains were found to inhibit the growth of known fish pathogenic bacteria or isolates that dominated the intestinal community of larvae in tanks with an overall poor success of larvae. A mixture of three selected strains was used for treatment of larvae and eggs in various treatment schedules. The numbers of cultivable bacteria were determined, as well as the total bacterial community structure of the larval intestines, using denaturing gradient gel electrophoresis of PCR amplified 16S rDNA. The bacteria were added to the egg incubators and first feeding larvae were fed enriched *Artemia franciscana*. The strains were found to establish themselves in the bacterial community of treated eggs but were not observed in the control group. Reduced incidence of jaw deformation was found amongst yolk sac larvae treated during the egg stage. All strains were identified as a part of the dominating intestinal community of treated larvae. Improved growth, survival and overall success of larvae was found in tank with treatment from the onset of feeding, compared to the control tank containing larvae of common origin

P6-4
Selection and identification of potential marine probiotic bacteria from *Dicologoglossa cuneata* (Moreau, 1881)

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Infectious diseases constitute a limiting factor in the development of the aquaculture production. The probiotic bacteria have been used as biological control agents in aquaculture and considered an alternative to the use of antibiotic to avoid the development of antibiotic resistance in fish pathogens. The aim of this study was to select, identify and characterise potential probiotic bacteria which were obtained from intestinal and skin mucus of fish and from homogenized larvae. Strains were selected according their ability to inhibit the growth of the most frequent fish pathogens: *Vibrio harveyi* (2 strains), *Photobacterium dam*selae subsp. *piscicida* (2 strains) and *Tenacibaculum maritimum* (one strain). Only 10 of 183 strains exhibited in vitro antibacterialactivity against these pathogens. The selected strains were

identified biochemically by API 20 NE and genetically by 16S rDNA sequence analysis. Additional tests were carried out in order to make a second selection among them using different methods: resistance to bile and growth, resistance and adhesion to intestinal and skin mucus. In future, research will be focused in the evaluation of pathogenicity of these selected strains to confirm that no pathogenic effects could occur in the host.

P6-5
Encapsulation of a bacterial fish probiotic in alginate beads: Protective effect under *in vitro* simulation of fish gastric conditions

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Probiotics confer health benefits in fish, but the survival after gastric transit in stomached fish remains a challenge for their effective action. In this work, alginate beads were prepared to encapsulate probiotic (PDP-11) with the purpose of enhancing their survival during exposure to different simulated fish gastric conditions (SFGC). Bacteria samples were suspended in 2% alginate and the beads were prepared extruding the suspension through a sterile syringe, and the resulting droplets were placed into a 2% $CaCl_2$ solution. The mean wet weight of the recovered beads was (sd, 2) mg, and the amount of viable cells encapsulated was 6.1 (sd, 0.3) (log CFU $bead^{-1}$). Both free and encapsulated cells were mixed with *Solea senegalensis* stomach extracts at different pH values, and samples were withdrawn from the mixture at 5, 60 and 120 min. Surviving bacteria after SFGC were enumerated by plate count. Free bacteria resisted to SFGC at pH 7, 6 and 5, and no significant decrease in survival after 2 h incubation was observed. In contrast, the number of cells declined drastically after 5 min at more acidic pH (5, 4 and 3). The encapsulation improved significantly the survival of cells at pH 4, since the bacteria remained totally viable during 120 min. At pH 3, this protective effect was noticed only during the first 5 min, and no protection was recorded when beads were incubated at pH 2. The encapsulation could be used to protect this probiotic in fish with gastric values > 4.

P6-6
Effect of alginate and calcium chloride on the encapsulation efficiency of a bacterial fish probiotics

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It is accepted that probiotics can improve the immunity and nutrition level of cultured fish becoming more resistant to infectious diseases. Successful colonization of viable probiotics in the intestine is essential for their efficacy. However, bacteria may not survive in sufficient numbers in fish food or during gastrointestinal passage. Thus, probiotic encapsulation constitutes an alternative to improve their viability. Influence of alginate (1, 2, 3 and 4%) and calcium chloride (0.5, 1, 2 and 3%) concentrations on encapsulation efficiency of a fish

probiotic bacterial strain (pdp11) has been evaluated. Bacterial suspensions in alginate were extruded into droplets using a 16-gauge needle falling into CaCl2 solutions. For all conditions encapsulation efficiency was higher than 70%. Highest percentages corresponded to capsules made with 0.5% CaCl2 and 1% alginate (90.1%) or 2% alginate (89.1%).Volume of alginate-bacteria drops before contact with CaCl2 was similar regardless of alginate concentration. However, capsule diameters ranged from 2.43mm to 3.13mm, higher sizes being obtained for higher alginate concentrations. Size differences may be attributed to a thicker coat of cross-linked calcium-alginate. Increasing calcium resulted in a decrease in encapsulation efficiency, probably due to higher resistance to disaggregation of these capsules. Implications on probiotics survival into fish food and on release during fish gastrointestinal passage need to be considered.

P6-7
Effect of probiotic bacteria on the protein expression in first feeding Atlantic cod (*Gadus morhua*) larvae

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Proteome analysis provides valuable information on the variations that occur within the proteome of organisms. These variations may for example reflect a response to biological perturbations or external stimuli resulting in different expression of proteins, post-translational modifications (PTMs) or redistribution of specific proteins within cells. The aim of our project was to use proteome analysis to create new knowledge on the effects of probiotic bacteria on protein expression in first feeding Atlantic cod (*Gadus morhua*) larvae. Newly hatched larvae were divided into 2 groups: C-group (Control), and P-group (group treated with probiotic bacteria). The survival rate was 5-fold higher in the P-group than in the C-group. Soluble larval proteins were analysed by 2 Dimensional Gel Electrophoresis and the profiles compared by computer analysis. In total, 430 protein spots were identified and their abundance monitored for both groups. Sixteen protein spots were significantly ($P<0.05$) induced in the P group compared to the controls and 59 spots were depressed. Proteins of interest were excised from stained gels and identified by peptide mass mapping. Alpha actins showed the most pronounced changes, where 4 different alpha- actin isoforms were increased in the P-group. The alpha -actins are found in muscle tissues and are a major constituent of the contractile apparatus. The different isoforms may be either encoded by different genes or generated by PTMs of the same gene product.

P6-8
Multiplex detection and identification of fish pathogens using DNA array technology

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Timely and reliable detection of fish pathogens is one of the limitations of fish disease management. Fish diseases can be caused by several organisms, incl. bacteria, fungi, viruses and protozoa. Consequently, identification of potential pathogens requires a diversity of time-consuming and laborious assays. The advent of molecular biology, in particular PCR, has opened alternative means for detection and identification of fish pathogens. Nevertheless, although most of these methods are convenient for the detection of one target, they are not suitable to simultaneously detect multiple pathogens. In contrast, DNA array technology may lead to unlimited multiplexing, i.e. detection and identification of numerous targets in a single assay. In this poster, we show the power of DNA arrays as a part of an innovative concept for the ornamental fish industry to simultaneously detect and identify a comprehensive set of fish pathogens. Each diagnosis can be achieved within 36 hours of sampling based on an objective technique utilizing an array of specific DNA fragments. Ultimately, the diagnostic kit will contain detector oligonucleotides for a diverse set of pathogens, ranging from prokaryotic and eukaryotic pathogens to viruses including multiple species from *Aeromonas*, *Flavobacterium, Mycobacteriu*m, *Vibrio* and Y*ersinia*, the fungus *Aphanomyces invadans* (EUS), and a selection of viral pathogens; Koi Herpes Virus, Spring Viremia of Carp Virus and Infectious Salmon Anemia Virus.

P6-9
Lymphoma in northern pike (*Esox lucius*) from the Archipelago Sea in Finland

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Lymphoma has been recorded from northern pike (*Esox lucius* L.) from the Baltic Sea since the 1970s. The tumours are usually scattered over the trunk of the fish but sometimes also on the head. The underlying musculature is usually unaffected (2). Histologically the tumours have been characterized as a histiocytic lymphoma (4). The pike lymphoma is suggested to be associated with a retrovirus infection. The presence of retroviruses in similar tumour tissue from pike has been reported from Ireland and North America (1, 3). Pikes with lymphoma were collected from the Archipelago Sea in spring and autumn. Standard transmission electronmicroscopy technique was used to examine the morphology of the tumour tissue collected at different seasons of the year and for screening for virus particles in the tumour

tissue. In a second part of this study RNA from the tumour tissue was isolated for running RT-PCR (reverse transcriptase-polymerase chain reaction). Primers for RT-PCR were designed based on conserved sequences of the genome of known retroviruses. The PCR product was cloned and sequenced. The sequence was compared with other known retroviruses.

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P6-10
A molecular approach to pre-harvest impact on post-harvest quality of trout

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An upcoming issue for the aquaculture production of tomorrow will be to link pre-harvest production history and post-harvest quality. The impact of pre-harvest production methods and history on quality of aquaculture products has been a field of limited investigation, despite industrial knowledge of major quality differences in raw material. Current advances in molecular biology offer possibilities to improve knowledge and develop models to evaluate pre-harvest muscle quality. Data, which elucidate linkage between history and post-harvest quality on a molecular level, will be presented from experiments involving such pre-harvest parameter as stress, feed and tissue damage. Furthermore the impact of such parameters will be related to sensory facts relating to consumer quality. Resent data in relation to diseases show that there is molecular evidence for a linkage between muscle regeneration post-infection and subsequent changes in eating quality of fish. For the future, this approach will give rise to unique tools capable of discriminating qualities of fish at a pre-harvest level and lead to a higher awareness of increased welfare in the aquaculture industry, as history would be an issue affecting quality and price.

P6-11
Effect of oxytetracycline and lisozyme dimer on morphological pattern of hepatocytes in Siberian sturgeon (*Acipenser baeri*, Brandt 1869)

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The Siberian sturgeons have survived till nowadays though they've been subjected to strong anthropopression and reduction of their population. Moreover, during the intensive breeding it becomes susceptible to the infectious diseases. The studies were carried out to estimate the effect of oxytetracycline (OTC) and lysozyme dimer (LD) on the morphology of the

hepatocytes in Siberian sturgeon. The experiment was carried out on 150 Siberian sturgeons of 2 400 g b.m. The fish were divided into 5 groups (1, 2, K1, K2, K3: n = 30). Fish from the gr. 1 and 2 were injected with 50 and 100 mg/kg b.m. i.p. of OTC and then bathed in water with addition of LD (100 μg/l) for 30 min, 24 hours after the injection. The fish from the gr. K2 – OTC (100 mg/kg b.m.), K3 – LD, a K1 – without these factors. Six fish from each gr. were slaughtered at the same time (directly after the bath in LD and after 3, 7, 14 and 21 days) and they were examined macroscopically and the livers were analyzed microscopically (HE, PAS). The use of sturgeon in the experiment allowed for the estimation of the influence of OTC at a dose recommended (50 mg/kg, b.m.) and dose increased (100 mg/kg b.m.) on the morphology of hepatocytes. The results also shoved the protective role of LD on the hepatocytes in sturgeons subjected to the action of suppressive doses of OTC.

P6-12
Detection and stimulation of IgM in first feeding cod larvae

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The presence of IgM and lysozyme in first feeding cod larvae was studied using specific antibodies made in rabbit and image analysis of cryosectioned larvae. IgM was detected in the gastrointestinal tract of larvae already at 28 days post hatch, and in higher intensities in the gut wall at 42 days post hatch. IgM has previously only been identified in approximately 50 days post hatch and further developed cod larvae. Lysozyme was commonly observed in the surface mucus but only sporadically in the gastrointestinal tract of larvae. This enzyme has been studied in juvenile cod but has, to our knowledge, not previously been studied in first feeding larvae. Feeding larvae peptide hydrolysates of pollock muscle proteins through the live feed, resulted in improved larval development, with organs and tissues markedly more distinguishable and defined in treated larvae compared to larvae that were not fed the peptides. Treatment furthermore induced higher intensities of IgM in the digestive tract and IgM was detected more widespread in the gut wall. Treatment also resulted in reduced incidence of malformation amongst larvae, with lower ratio of larvae developing curved notochord and other deformities commonly observed among larvae at transfer to weaning.

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