SCOFDA workshop

Diagnosis and Control of Fish Diseases

Main themes:
Control of Pathogens in Warm Water Aquaculture and Recirculated Model Trout Farms

November 4 and 5, 2008
10.00-17.00

Venue:
Lecture hall 1-01 (November 4)
Lecture hall 2-01 (November 5)

University of Copenhagen, Faculty of Life Sciences
Bülowsvej 17
DK-1870 Frederiksberg C
Denmark

Programme and abstract book
Edited by Kurt Buchmann

Organised by the research school
SCOFDA
Sustainable Control of Fish Diseases in Aquaculture
University of Copenhagen
Faculty of Life Sciences

Kurt Buchmann KU-Life (leader)

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Program printed by Frederiksberg Bogtrykkeri
October 2008

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SIXTH FRAMEWORK
PROGRAMME

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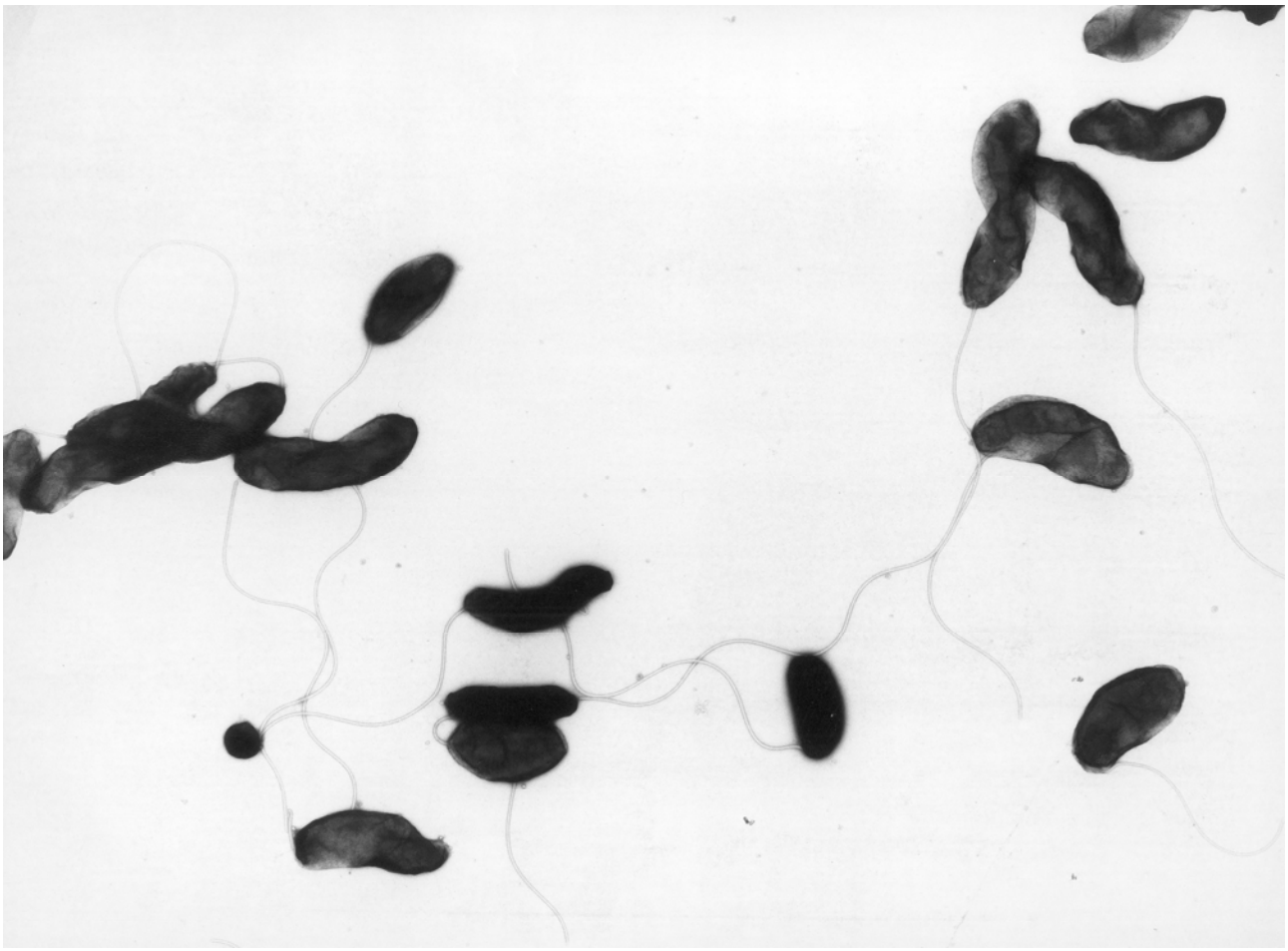

Photo: *Vibrio anguillarum* bacteria, important pathogens in marine fishes. Courtesy to J. L. Larsen and B. Bloch

Programme

November 4, 2008
Lecture hall 1-01, Bülowsvej 17

10.00-10.45
Dr. Barbara Nowak, University of Tasmania, Australia
Recent developments in disease control Down Under

11.00-11.30
Dr. Kjartan Hodneland, University of Valencia, Spain
IPNV in glass-eels from the Iberian Peninsula

11.30-12.00
Dr. Katja Einer-Jensen
Identification of virulence markers in marine VHSV and use in diagnostics for aquaculture

12.00-12.45
Lunch at Gimle

13.00-13.30
Dr. Karl Pedersen
***Photobacterium damselae*: an emerging pathogen in the aquatic environment**

13.30-14.00
MSc Jakob Skov
Nematode larvae of wild and cultured fish

14.00-14.30
Coffee break

14.30-15.00
Dr. Lars Holten-Andersen
Principles in vaccines for mammalian hosts and their application in fish vaccines

15.00-15.30
Dr. Martin K. Raida
Long-term protection against ERM following immersion vaccination

15.30-15.45
Dr. Alf Skovgaard
Pathogens in crustaceans

15.45-16.00
Dr. Erol Toksen
The parasites of cultured common dentex

16.00-16.15
Dr. Lone Madsen
Diseases of mussels and oysters
Recent developments in diagnosis and control

16.15-16.45
Dr. Barbara Nowak
Amoebic gill disease (AGD) outbreaks in mariculture

16.45-17.00
Dr. David Verner-Jeffreys

Redmark syndrome/Coldwater strawberry disease of rainbow trout

17.00-17.15
Discussion and concluding remarks on the first day presentations

18.00-21.00
Dinner at Radisson SAS, Falkoner Centre restaurant, Falkoner Alle 9, 2000 Frederiksberg

November 5, 2008

NB: change of lecture room: Lecture Hall 2-01, Grønnegaardsvej

10.00-10.30
Dr. Barbara Nowak
How healthy are farmed southern bluefin tuna

10.30-11.00
M. Sc. Karl Iver Dahl-Madsen
The future for recirculated systems in fishfarming

11.00-16.00
Focus on MMS and recirculated trout farms

11.00-11.30

MSc Thomas Rohde Jørgensen
Parasitic infections in model trout farms

11.30-12.00
Dr. Niels Jørgen Olesen
Model trout farms: surveys of viral and BKD infections

12.00-13.00
Lunch at Gimle

13.00-13.30
Dr. Inger Dalsgaard
Bacterial diseases in model trout farms

13.30-14.00
DVM Niels Henrik Henriksen
Model trout farms from a vet's perspective

14.00-14.30
Coffee break

14.30-14.45
Niels Lorenzen
DNA vaccines and other molecular tools in fish health research

14.45-15.00
Keith R. Jeffery
Tilapia farming in England and Wales and the occurrence of *Franciscella* sp.

15.00-15.15
Yusüf Güner
Sustainable trout farming in Turkey

15.15-15.30
Gurel Turkmen
Integrated trout and crayfish culture in Turkey

15.30-16.00
Discussion and conclusion of the workshop

ABSTRACTS

Recent developments in disease control Down Under

Barbara F. Nowak

National Centre for Marine Conservation and Resource Sustainability, AMC Institute, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania and Aquafin CRC, Launceston, Tasmania, Australia

Mariculture in Australia covers a wide range of fish species. Southern bluefin tuna and Atlantic salmon are the main two species cultured in seapens, there are also commercial companies growing barramundi, snapper, yellowtail kingfish and new species for aquaculture including striped trumpeter, dhufish and mulloway are being investigated. This large number of species provides an interesting challenge for fish health researchers as well as vaccine manufacturers. Infectious disease can cause significant problems in fish culture. Immunomodulation, enhancing immune response by diet and use of immunostimulants, vaccines and adjuvants, is often required to continue sustainable (both environmentally and economically) fish production. Many viral and bacterial diseases which are the main concern for aquaculture in Northern Hemisphere are absent from Australia. Only a few vaccines, mostly bacterins are currently used. Traditionally they were all applied as immersion vaccination, but recently injection vaccination is becoming more popular. This presentation will summarise main ways to improve immune response in cultured fish in Australia, including vaccination, use of immunostimulants and adjuvants. The examples used will be based on recent research at the University of Tasmania, including work on vaccines and adjuvants for Atlantic salmon, *Salmo salar*, vaccines and adjuvants and vaccines for barramundi, *Lates calcarifer* and immunostimulants for snapper, *Pagrus auratus*. Potential for immunomodulation to control amoebic gill disease in salmonids will be discussed. New research plans for improving efficacy of

vaccines will be presented. Improved knowledge and understanding of parasitic conditions will result in better control and treatment methods, thus increaseing profitability and sustainability of mariculture.

Recurrent isolation and detection of infectious pancreatic necrosis (IPN) virus from glass–eels originating from the Iberian Peninsula.

Hodneland, K[1], A. Montesinos[2], C. Sexton[1], E. Sanjuán[2] and C. Amaro[2]

1 Unidad de Zoología Marina, Instituto Cavanilles de Biodiversidad y Biología Evolutiva.

2 Departamento de Microbiología y Ecología, Universidad de Valencia.

The eel-farming industry relies heavily on the supply of juvenile fish from catches of wild glass-eels. The glass-eels are caught in the estuaries and river-mouths before transported to the farm where they are fed to market size or for restocking. In recent years, a local eel-farm in Valencia (Spain) has experienced high mortalities during the first month after the arrival of glass-eels. Clinical signs include spiral swimming and/or un-coordinated behaviour, with some haemorrhages in the gills and viscera. The isolation and partial characterization of an infectious pancreatic necrosis (IPN) virus from affected glass-eels will be presented. This is the first report of IPNV from eel in Spain. In order to check the IPNV-status of glass-eels before and after arrival in the eel-farm, a sensitive and specific real-time RT-PCR assay was developed and optimized. A batch of glass-eels was screened weekly (0-6 weeks) for the presence of IPNV. The results demonstrate that the glass-eels were already infected with the virus at the time of arrival in the farm plant. Highest virus load as measured by the real-time RT-PCR assay was observed only days before the peak mortality.

Identification of virulence markers in marine VHSV and use in diagnostics for aquaculture.

Katja Einer-Jensen* and Niels Lorenzen

National Veterinary Institute, The Section for Fish Diseases,Technical University of Denmark

Production of rainbow trout in marine aquaculture is an expanding industry in Denmark, but a circulating reservoir of the fish pathogenic viral haemorrhagic septicaemia virus (VHSV) in wild fish populations represents an important threat towards the future success of these activities.

The Danish Research Council for Technology and Production Sciences (FTP) has recently funded this project which aims at:

- Understanding the biological background for development of the disease (VHS) in fish.
- Identification of genetic markers for virulence of VHSV isolates in rainbow trout.
- Development of a diagnostic tool for rapid virulence-typing of VHSV isolates.

Various molecular tools will be applied such as reverse genetics for generation of recombinant virus, *in vivo* imaging and immunofluorescense for *in situ* pathogenesis studies as well as virulence testing in small scale animal experiments. The expected result will establish an extended platform for new research into pathogen virulence and host immunity, which will have both applied (disease prophylaxis in aquaculture) and basic (general understanding of disease and immunity) perspectives. A PhD Scholarship in Molecular Biology at the National Veterinary Institute (DTU VET) is available in relation to this project, starting date December 2008.

Application deadline is October 30st 2008.

Photobacterium damselae subsp. d*amselae*, an emerging pathogen in Danish marine rainbow trout

Karl Pedersen[1,2], Helle Frank Skall[2], Anne Marie Lassen-Nielsen[2], Lotte Bjerrum[2], Niels Jørgen Olesen[2]

[1]University of Copenhagen, Faculty of Life Sciences, Department of Veterinary Pathobiology, Stigbøjlen 7, DK-1870 Frederiksberg C, Denmark, [2]National Veterinary Institute, Technical University of Denmark, Hangøvej 2, DK-8200 Århus N, Denmark

Recent investigations have demonstrated that *Aeromonas salmonicida* subsp. *salmonicida* and *Vibrio anguillarum* are important bacterial pathogens in Danish rainbow trout mariculture. However, investigations also suggested that *Photobacterium damselae* subsp. *damselae* played a bigger role than previously assumed. This bacterial species has mainly been described as a cause of mortality in turbot and other fish species in the Mediterranean and it may also be pathogenic to mammals, including humans, but we have very sparse knowledge about this bacterium in Northern Europe. *P. damselae* subsp. *damselae* prefers warm water, and in light of the anticipated increases in water temperatures as a consequence of global climate changes, disease problems caused by this organism can be expected to increase. At present there is no vaccine against *P. damselae* subsp. *damselae* whereas a commercial vaccine is available against the closely related *P. damselae* subsp. *piscicida*. Therefore it would be appropriate to study in more detail the properties of this bacterium in order to evaluate and improve principles for therapy and prophylaxis. The present study was undertaken to investigate phenotypic and genotypic properties together with virulence of a collection of 16 clinical isolates of *P. damselae* subsp. *damselae*, recovered in a recent survey from diseased rainbow trout. Their properties with respect to cell and colony morphology, odour, catalase and oxidase reaction, Gram properties, and haemolysis were recorded and supplemented with

biochemical tests. Haemolysis was measured as the diameter of the haemolysis zone on blood agar recorded after incubation for 48 h at 20 °C. Genotyping was performed by pulsed-field gel electrophoresis using *Not*I as restriction enzyme, where after similarity between banding patterns was calculated by the dice coefficient followed by clustering using the UPGMA method. Four isolates, two displaying a broad zone of haemolysis and two showing a narrow zone of haemolysis, were investigated for their pathogenicity to rainbow trout as judged from LD_{50} values. Test for antimicrobial susceptibility was carried out by the disk diffusion method and a representative for the major antimicrobial classes was selected: florfenicol (amphenicols), oxolinic acid (quinolones), ampicillin (extended-spectrum penicillins), oxytetracycline (tetracyclines), neomycin (aminoglycosides), colistin (polymyxins), sulphamethoxazole (sulphonamides), and trimethoprim. In addition, the isolates were tested for susceptibility to the vibriostatic agent, O/129. All isolates belonged to the subspecies *damselae,* being positive in urease, haemolysis and motility. All isolates were also positive in catalase, oxidase, arginine hydrolase, and fermentation of glucose, but negative in β-galactosidase, ornithine decarboxylase, H_2S, tryptophane deaminase, indole, and fermentation of mannitol, sorbitol, rhamnose, and arabinose. Few isolates were positive or weakly positive in citrate, Voges-Proskauer, gelatinase, saccharose, melibiose, or amygdalin. Six of the isolates were positive in lysine decarboxylase although the change of colour of the pH indicator in the growth medium was not as pronounced as for arginine dihydrolase. All isolates were sensitive to florfenicol, oxolinic acid, oxytetracycline, colistin, and neomycin. One isolate was resistant to ampicillin. Five isolates were resistant to trimethoprim, and these six isolates were additionally resistant to sulphonamides and – as the only ones – to the vibriostatic agent, O/129. Most of the isolates (14/16) were resistant to sulphonamides, while the remaining two isolates were intermediately susceptible. The most often used antimicrobials in Danish marine aquaculture are potentiated sulphonamides. It may therefore be suggested that whenever problems with *P. damselae*

subsp. *damselae* occur, oxolinic acid should be used instead. The connection between resistance to trimethoprim and O/129 has previously been recorded for other marine bacteria, but the genetic background is not known. The PFGE results revealed the existence of several different profiles, which indicated that the disease problems were not caused by the introduction of a single virulent clone, but by the occurrence of several clones simultaneously. However, the isolates grouped in two clusters, which turned out to differ in their haemolytic properties: the average diameter of the haemolysis zones of cluster 1 and 2 were 38.9 mm (SD=10.8 mm) and 23.5 mm (SD=6.0 mm), respectively, which was statistically significantly different in a t-test (p=0.002). Two strains with strong haemolytic properties and belonging to PFGE cluster 1 and two strains with weak haemolytic properties and belonging to PFGE cluster 2 were then used in challenge studies to establish their LD_{50}. Virulence of the strains to rainbow trout was highly variable but the results showed a very strong relation between haemolysin and virulence to fish as there was a difference in LD_{50} between the cluster 1 and cluster 2 strains of about 10,000 times (Table 1). This suggests a strong involvement of haemolysin in the pathogenesis, but also that there are two populations of *P. damselae* subsp. *damselae*: a virulent and a non-virulent one. One strain was tested for virulence at both 13 °C and 20 °C, and the result was very clear: the bacterium was much more virulent at 20 °C, LD_{50} being more than 1000 times higher at 13 °C than at 20 °C. This explains why mortality among rainbow trout in marine aquaculture due to *P. damselae* subsp. *damselae* only occurs during periods with high water temperatures. Pathological changes were indicative of a bacterial haemorrhagic septicaemia. A dominant pathological finding was extensive haemorrhages, which were located mainly in the skin, around the vent, in the parietal peritoneum, and the intestinal tract, in particular its aboral segments. Skin haemorrhages were most pronounced at fin bases and along the ventral midline of the abdomen. A serohaemorrhagic ascites fluid was present in the abdominal cavity of all fish. Petecchial haemorrhages were also often observed in the liver. At present we have

no knowledge about the prevalence and distribution of *P. damselae* subsp. *damselae* in Danish coastal waters, and we have no knowledge of seasonal fluctuations. It therefore seems urgent that quantitative spatial-temporal investigations are carried out.

Table 1. Relation between water temperature, haemolytic activity, and virulence of *P. damselae* subsp. *damselae*.

Isolate number	Water temperature, °C	Haemolysis zone, mm	LD_{50}, cfu
206328-2	13	45.2	5.2×10^{6}
206328-2	20	45.2	3.9×10^{3}
206308-4	20	54.5	3.6×10^{4}
206276-1	20	15.0	1.5×10^{8}
206303-1	20	19.1	1.5×10^{7}

Nematode larvae in wild and cultured fish

J. Skov, P. W. Kania, M. M. Olsen, J. H. Lauridsen and K. Buchmann

Department of Veterinary Pathobiology, Section of Fish Diseases, Faculty of Life Sciences, The University of Copenhagen, Frederiksberg C, Denmark

Nematodes of the family Anisakidae are parasites of marine mammals. Life cycles include in most cases both crustaceans and fish. The larval stage of anisakid nematodes found in the fish host may attempt to penetrate the stomach and intestinal wall of man if viable larvae are accidentially ingested along with an inadequately processed fish meal. Symptoms resulting from this may be stomach ache, nausea and vomiting. The human pathogenic 3rd stage larvae of *Anisakis simplex* is

well known to be present in squids and in the flesh of a wide range of marine fish species including wild salmonids. However, studies conducted in Norway, Scotland, USA and Japan have shown that salmonids reared in marine net cages are free from infection with larval anisakids. In the present study rainbow trout (*Oncorhynchus mykiss*) from a Danish mariculture facility were examined for the presence of nematode larvae in the body cavity by dissection (*n*=166) and in the flesh of the belly flaps by pepsin digestion (*n*=106). No nematode larvae were found in cultured *O. mykiss*. In contrast, wild fish from natural populations were found infected. Thus, for comparison, ten specimens of each of 15 commercially important fish species caught in their natural habitat in Danish waters were included in the study and examined as described above. Nematode larvae found in these wild caught fish species were diagnosed by morphological identification and by PCR and sequencing of the ITS1 region. *Anisakis simplex* third stage larvae were found to be present in wild fish populations. The background for the parasite-free status of the Danish maricultured rainbow trout is due to feeding with parasite free commercial feed pellets (heat treated, extruded) whereby the cultured fish remain un-exposed to infective parasite larvae.

Principles in vaccines for mammalian hosts and their application in fish vaccines

Lars Holten-Andersen

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Laboratory of Fish Diseases, Department of Veterinary Pathobiology

Faculty of Life Sciences, University of Copenhagen

Despite various differences between immune systems of fish and mammals it is apparent that many basic vaccine principles developed for the latter can be applied in the fish farming industry. These include type of vaccine (e.g. whole-cell or sub-unit) and design of vaccine formulation (i.e. choice

of antigen and adjuvant). However, when it comes to vaccination strategies that rely on the effect of booster injections one must consider the practical problems associated with recapturing and handling of farmed fish. These difficulties might compromise the application of such protocols, although the answer could be boosters delivered in the food. Hence, numerous variables have to be considered when new vaccine experiments are planned, some of them related to the experimental model (fish species, vaccination/challenge interval, challenge/necropsy interval, challenge dose, route and bacterial strain), others more related to the vaccine composition in terms of antigen (source of antigen and dose) and adjuvant (choice of vehicle and relevant immunomodulators). These points are all taken into consideration in our design of novel vaccines to protect rainbow trout, *Oncorhynchus mykiss*, from infections with *Aeromonas salmonicida*, *Vibrio anguilarum* and *Yersinia ruckeri*. We will build upon the knowledge from vaccination trials in mammals, since this field in many aspects is ahead of vaccine developments in the fish farming industry. Our major goal is to match or improve the level of protection induced by existing vaccines against the above-mentioned pathogens while reducing the side effects that are currently found in many of the vaccinated fish. This talk will cover the fundamental steps of our strategy.

Long term protection of immersion vaccinated rainbow trout against *Yersinia ruckeri* infection.

Martin K. Raida[1] and Jørgen Nylén[2]

University of Copenhagen, Frederiksberg, Denmark[1]

Schering-Plough, Ballerup, Denmark[2]

Studies have been conducted on the long term effect of immersion vaccination of rainbow trout against *Yersinia ruckeri* O1, the bacterial pathogen causing enteric red mouth disease (ERM). Protection of rainbow trout following immersion-vaccination with a commercial vaccine

(AquaVac^{TM} ERM vet. Schering Plough Animal Health) and an experimental bacterin of *Yersinia ruckeri* O1 was determined. A total of 1200 rainbow trout were divided into groups which were immersion-vaccinated with either the commercial or the experimental bacterin. The control groups were sham vaccinated by immersion in pure water. Sub-groups were challenged by immersion into a bacterial suspension ($1x10^9$ CFU *Y. ruckeri*/ml, LD_{50} dose) two month post-vaccination. Both vaccinated groups showed 100% survival which was highly significant compared to the un-vaccinated controls (40% mortality) ($P<0.0001$). Four month after immersion-vaccination half of the rainbow trout vaccinated with AquaVac^{TM} ERM vet. received an oral booster vaccine (AquaVac^{TM} ERM Oral vet) (two weeks feeding according to the manufacturer's instructions). Six month post-vaccination other sub-samples of fish from all groups were bath-challenged. There was still found 100% survival in all of the vaccinated groups which was a significantly higher survival compared to the un-vaccinated controls (28% mortality) ($P<0.0001$). Plasma samples from fish were taken during the experiment. The bactericidal effect of plasma from both vaccinated and un-vaccinated fish were tested against a 10-fold dilution series of live *Y. ruckeri*. Following 1 hour incubation the samples were plated onto blood-agar. The bacterial count was much lower in plasma samples from vaccinated rainbow trout compared to un-vaccinated control fish. This result indicates the presence of one or more components in plasma which are able to reduce the growth or even kill *Y. ruckeri*. These factors are up-regulated following immersion-vaccination and are likely to be associated with increased survival of the vaccinated rainbow trout. Future ELISA analysis of the plasma samples will elucidate if immersion vaccination and the oral booster vaccination has had an effect on the titre of *Y. ruckeri* specific antibodies. The present experiment has documented 100% protection of immersion-vaccinated rainbow trout for up to six month post vaccination.

Pathogens in crustaceans

Alf Skovgaard

University of Copenhagen, Faculty of Life Sciences, Department of Veterinary Pathobiology

Section of Fish Diseases

Many species of parasitic dinoflagellates exist, but only a few of these have been studied in much detail. Some species are known to cause severe infections in fish (e.g. *Amyloodinium*) as well as in fish eggs and larvae (*Ichthyodinium*). Also crustaceans are infected by parasitic dinoflagellates. The genus *Hematodinium*, comprising a so far unrecognized number of species, infects a wide array of commercially important crustaceans such as *Nephrops norvegicus*, *Cancer pagurus*, *Callinectes sapidus*, *Chionoecetes opilio*, and others. It appears that a single species – or phylotype – is present in many different host species in the entire North Atlantic coastal area. Reported effects in these hosts vary from virtually no effect to large scale declines of crab populations. Closely related parasitic dinoflagellates of the genera *Syndinium* and *Blastodinium* infest copepods and have a significant effect on populations of these small, plantonic crustaceans that dominate the oceans zooplankton. I will present a brief overview of these pathogens in crustaceans and present some recent findings on their effects, distribution, and prevalence.

Amoebic Gill Disease (AGD) outbreaks in fish mariculture

Barbara F. Nowak

National Centre for Marine Conservation and Resource Sustainability, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, and Aquafin CRC, Locked Bag 1370, Launceston Tasmania 7250 Australia

Amoebic Gill Disease (AGD) has been reported from a number of farmed fish species worldwide. The disease manifests in severe alterations of gill tissues. Epithelial hyperplasia leading to lamellar

fusion is associated with inflammatory changes in the gills. AGD can increase fish production costs due to the cost of treatment or, due to direct fish losses. AGD is the most significant health problem affecting Atlantic salmon culture in Tasmania Australia. Fish with AGD develop multifocal gross gill lesions as a result of epithelial hyperplasia. Current treatment for AGD is freshwater bathing, the frequency is decided on the basis of gross gill checks. This disease has also been recorded from other countries (USA, Ireland, France, Spain, Scotland, Norway, Chile, New Zealand) and other cultured fish species (rainbow trout, coho salmon, turbot). While initially it was assumed that *Neoparamoeba pemaquidensis* is responsible for AGD outbreaks, in situ hybridisation study covering archival samples from a number of fish species and locations showed that the only species of amoeba associated with AGD gill lesions is *Neoparamoeba perurans*. Transcriptome profiling of gill lesions in Atlantic salmon experimentally infected with AGD showed that transcripts associated with the immune response were almost universally down-regulated. In AGD-affected tissue significant, coordinated down-regulation of the major histocompatibility complex class I (MHC I) pathway-related genes occurred during the later stages of infection. Within this micro-environment, suppression of the MHC I and possibly the MHC II pathways may inhibit the development of acquired immunity and could explain the unusually high susceptibility of Atlantic salmon to AGD.

The parasites of cultured common dentex (*Dentex dentex* L.)

Erol Toksen*, Esat Çilli

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The parasite fauna of cultured common dentex from the Aegean sea coast of Turkey was investigated in the period between May and October 2007. A total of 94 fish were examined and

five protozoan and three metazoan parasite types were found. The parasites recovered comprised *Amyloodinium ocellatum* Brown and Hovasse, 1946; *Trichodina* Ehrenberg, 1838; *Epistylis* Ehrenberg, 1830; *Riboscyphidia* Jankovski, 1985; *Ceratomyxa* Thelohan, 1892; *Gyrodactylus* Nordmann, 1832; *Microcotyle* Beneden and Hesse; *Clavellotis* Castro and Baeza, 1984. The findings are discussed with special emphasis on possible pathological effects on the host.

Coldwater strawberry disease/Red mark syndrome of rainbow trout (*Oncorhynchus mykiss* Walbaum)

David. W. Verner –Jeffreys

Cefas, Weymouth laboratory, The Nothe, Barrack Road, Weymouth, Dorset, UK

Coldwater strawberry diseases (CWSD), also known as Red Mark Syndrome, is a skin disease that has emerged in farmed rainbow trout in the UK. CWSD has severe commercial impact, as affected fish are downgraded at harvest. Epidemiological investigations and transmission studies in our laboratory have shown this likely has an infectious aetiology. This presentation will provide a summary of the information we have to date about this important condition, including the results of continuing efforts to uncover the responsible aetiological agent.

Reference: Verner-Jeffreys DW, Pond MJ, Peeler EJ, Rimmer G, Oidtmann B, Way K, Mewett J, Jeffrey K, Bateman K, Reese RA and Feist SW (2008) Emergence of coldwater strawberry disease of rainbow trout, *Oncorynchus mykiss* Walbaum in England and Wales: outbreak investigations and transmission studies. Diseases of Aquatic Organisms 79: 207-218

How healthy are farmed Southern Bluefin Tuna?

Barbara F. Nowak

National Centre for Marine Conservation and Resource Sustainability, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, and Aquafin CRC, Locked Bag 1370, Launceston Tasmania 7250 Australia

Southern Bluefin Tuna (*Thunnus maccoyii*) has been farmed off Port Lincoln South Australia since early 1990s. Wild 2-4 year old Southern Bluefin Tuna are captured in Great Australian Bight and towed to the farming sites where they remain for 3-8 months. During this time the tuna are fed fresh and frozen baitfish. While there are no health issues affecting Southern Bluefin Tuna, a project was established to determine baseline for health of this species under farming conditions. Fish health can be measured either by determining normal ranges for healthy individuals - for example for blood variables - or by observing presence of parasites or pathology in fish. Normal parasite loads on a farmed Southern Bluefin Tuna were investigated. Other methods to determine Southern Bluefin Tuna health such as measurements of innate immune response variables, hemoglobin level, differential count of leucocytes have also been included. Their main advantage is the potential for non-lethal blood sampling, whereas most parasitic and histopathology investigation require killing of the fish. Most husbandry procedures did not appear to affect Southern Bluefin Tuna health as measured by parasite loads. Sampling year (possibly related to the cohort and environmental conditions) and company (possibly related to tow, location of cages, feeding and other husbandry procedures) showed some effect on parasite loads. However, the parasite loads were low and did not affect condition factors and other measured variables, such as hemoglobin levels. This investigation confirmed that farmed Southern Bluefin Tuna are healthy and that husbandry procedures, including holding over summer did not affect their health status.

Parasitic infections in model trout farms

Thomas R. Jørgensen, Thomas B. Larsen, Kurt Buchmann

Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen

Parasite infections in recirculated rainbow trout (*Oncorhynchus mykiss*) farms were monitored through 22 months. Parameters such as temperature, mortality, pH, nitrite and ammonia-concentrations, use of formalin, mortality and feed conversion rate were also monitored. Due to introduction of rainbow trout from traditional earth ponds into the new systems, all farms were found to be infected with a number of parasitic organisms known from traditional farming. In some farms white spot disease caused by *Ichthyophthirius multifiliis* was associated with high fish mortality. Furthermore, *Trichodina* spp., *Apiosoma* sp., *Ambiphrya* sp., *Epistylis* sp., *Chilodonella piscicola*, *Icthyobodo necator, Spironucleus salmonis, Gyrodactylus derjavini* and the eye fluke *Displostomum spathaceum* were recorded. Findings indicated that seasonal fluctuations of parasite populations are partly masked by the effects of chemical treatment and a less variable temperature level throughout the year. Thus, stabile and low temperature of supplying well water resulted in lower fluctuations of parasite infections compared to traditional farms using river water. The study underlines the problems caused by introduction of infected fish from traditional earth ponds into recirculated systems. The study frames production advantages of using pathogen free fish for stocking in modern farms aiming to achieving pathogen-free production feasible.

Rainbow Trout Farms with a High Degree of Recirculation: Are Pathogenic Bacteria a Problem?

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Recirculating systems combining increased production of rainbow trout (*Oncorhynchus mykiss*) farms and reduced environmental impact are progressing in Denmark. Eight traditional flow-through farms have been redesigned to "model farms" based on recirculation technology. To improve the Danish know-how in relation to disease problems at fish farms with a high degree of recirculation the occurrence of the well-known fish pathogens *Flavobacterium psychrophilum, Yersinia ruckeri* and *Aeromonas salmonicida* was studied. Bacteriological examinations of 20 fish (of different sizes) were done on each model farm quarterly. Samples were taken from skin, gills, spleen, kidney, brain and pathological changes if present and inoculated on blood agar (for the isolation of *Y. ruckeri* and *A. salmonicida*) and tryptone yeast extract salts agar for the isolation of *F. psychrophilum*. It was possible to isolate all three bacterial species, both from fish with and without disease symptoms. *F. psychrophilum* were isolated from all 8 farms, *Y. ruckeri* from seven of the eight farms, whereas *A. salmonicida* was only isolated from a few farms. Our results show that pathogenic bacteria can be found on the model farms, however, it seems that disease outbreaks caused by bacterial infections are reduced compared with traditional freshwater farms. The antimicrobial susceptibility of the isolated bacteria showed that all isolates of *Y. ruckeri* and *A. salmonicida* were susceptible for Tribrissen and oxolinic acid. More than 50% of the isolated *F. psychrophilum* were examined and all found susceptible for the therapeutic agent florfenicol. *F. psychrophilum* was the pathogen isolated from most of the examined fish mainly from gills and skin

mucus, but also from ulcers and internal organs. The significance of the occurrence of *F. psychrophilum* without simultaneous disease outbreaks is unclear. There were, however, indications that this bacterium might cause disease outbreaks during winter time. The results obtained in the monitoring period yielded an important basis for studies to further improve our knowledge of infections caused by *F. psychrophilum*.

Model trout farms from a vet's perspective

Niels Henrik Henriksen

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The recirculated model trout farms have illustrated that rainbow trout production in Denmark can be conducted in a relatively sustainable form without loss of profitability. Water intake from natural rivers is unnecessary due to use of well and drainage water. In addition, effluents to the environment have been reduced and the feed quota and thereby the production can be increased in these new fish farm systems. A number of disease problems have been encountered during the first years with the model trout farms. These were all known from earth ponds used in traditional trout farming but due to the special conditions in recirculated systems some diseases have dominated and attracted special attention. In order to cope with these challenges new systems for disease control have been implemented in the model trout farms. Some pathogens have been of less importance and others have challenged the fish health consultants to some extent. The present lecture outlines the main experiences obtained by the veterinary consultants during the first years of production. Special focus has been placed on white spot disease, PKD, BKD and ERM.

"DNA vaccines and other molecular tools for functional studies of fish-pathogen interactions *in vivo"*

Niels Lorenzen

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The rapid advance in full genome sequencing and gene array technology represents a major advance in our ability to analyse host-pathogen interactions at a molecular level. However, to fully understand the functional aspects, in vivo studies are equally important. Recombinant DNA technologies have here provided some very powerful tools. DNA vaccines encoding the glycoproteins of fish rhabdoviruses like VHSV and IHNV have proved very efficient under experimental conditions and can induce long-lasting protective immunity when delivered by intramuscular injection in rainbow trout. Immunity is established already a few days after vaccination. In this early phase protection is non-specific and related to interferon induced defense systems whereas specific antibodies and cellular components both play a role in the long-lasting protection. One explanation for the high efficacy of these vaccines thus seems to be the ability of the expressed viral glycoprotein to trigger both innate and adaptive immune mechanisms in manner comparable to what happens during a natural virus infection. The protective capacity of antibodies can be demonstrated in passive immunization experiments, which in the same time are very useful for identification of protective antigens. Cloning and manipulation of antibody genes provides further insight to the underlying mechanisms. Similarly, for understanding pathogenesis, recombinant pathogens carrying reporter genes represents a very potent technology, allowing *in vivo* monitoring of an infection. In terms of understanding the role of both host and pathogen molecules during infection, RNA interference technology has high potential for functional knock down studies, but the concept still has to be optimised for *in vivo* use in fish.

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Tilapia farming in England and Wales and the emergence of *Franciscella* sp.

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The last decade has seen the development of a small but growing Tilapia farming industry within England and Wales. The farms consist of various designs of re-circulation systems and have been primarily orientated towards agriculture farmers wishing to diversify. My talk will outline the development of the industry and the emergence of *Franciscella sp*. I will briefly detail a case study of the infected farm, the diagnostics, the actions taken by the fish farmer and possible implications for the industry.

Sustainable Trout Farming in Turkey

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The trout farming industry in Turkey has expanded considerably since 1969. The production in 2006 reached a total of 56,026 metric tonnes. Family and individual small scale farms producing 30 metric tonnes per year are the main contributors to the trout market. More advanced and large scale (1000 metric tonnes/year) integrated facilities have been established in recent years. The number of natural, artificial lake and cage farming has also increased. Cage culture in the Black sea has been limited to certain periods of the year due to high of water temperatures during summer time. However, new techniques and modern technology can support intensive cage farming in the Black

sea which in the future can obtain a strategic importance in trout farming. Trend analyses for the Turkish trout production indicate that a production of 100,000 tonnes per year will be feasible in 2020. Most trout farms are not using their full capacity and freshwater resources in various parts of Turkey have not yet been utilised for trout production. The predicted increase in aquaculture may cause adverse effects on ecology and environment. Some environmental effects due to trout farming have already been observed today. This report indicates some solutions for problems associated with the future development of trout farming in Turkey.

Integrated Trout-Crayfish Culture in Turkey

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The rainbow trout (*Oncorhynchus mykiss*) has been cultured in Turkey since 1969 and Turkey has become one of the top trout producing countries in Europe with an annual production of 56,000 tonnes amounting to 43 percent of the Turkish aquaculture production. *Astacus leptodactylus*, the narrow-clawed crayfish (popular name ‘‘Turkish crayfish’’), is the native freshwater crayfish species in Turkey. Due to overfishing, pollution and the crayfish plague (*Aphanomyces astaci*), the total annual harvest of *A. leptodactylus* became dramatically reduced from 5000 to 200 tonnes after 1985. However, in recent years (1996-2004), there has been a gradual increase of production. Aquaculture is important not only for food supply but also for purposes of restocking (including endangered species) and recreational fisheries. This presentation describes a project which can reduce the nutrient outflow from trout farms. The basic construct is an aquatic ecosystem consisting of one or several water bodies comprising an integrated food web. Horizontal integration of trout and crayfish aquaculture represents a technically viable opportunity for aquaculture producers to

reduce environmental impacts and enhancing production efficiency. The present talk outlines the basic principles in this integrated system.

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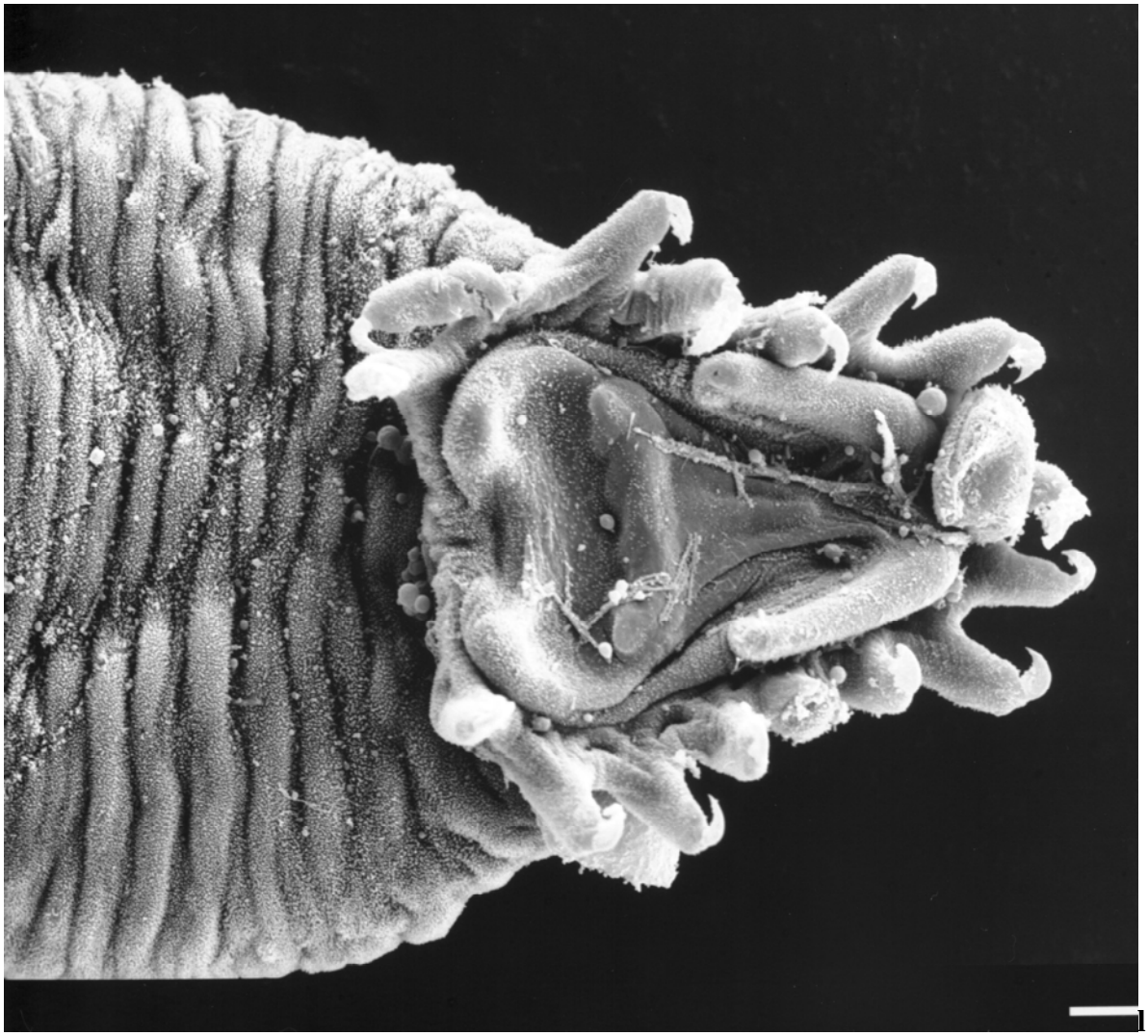

Gyrodactylus dejavinoides **from farmed rainbow trout. Photo by K. Buchmann and J. Bresciani**