Abildgaard Workshop
COST action 867 training School

Welfare of farmed fish – Nutrition, Breeding and Diseases

Date: April 11 and 12, 2007

Time: 10.00 to 17.00 both days

Venue:
Auditorium 1-01 (Fest-auditorium)
University of Copenhagen
Faculty of Life Sciences
Bülowsvej 17, DK-1870 Frederiksberg C.
Denmark

Organised by

SCOFDA

Sustainable Control of Fish Diseases in Aquaculture
Research School

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Supported by the
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Proceedings of the workshop and training school

Welfare of farmed fish – nutrition, breeding and diseases

Editor and organiser:

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Front cover: Peter Christian Abildgaard was the founder of the Royal Veterinary and Agricultural University (now from January 2007 the Faculty of Life Sciences at the University of Copenhagen). This was the first veterinary school in Scandinavia. He founded our university in 1773 and contributed to a long range of subjects including fish parasitology.

Picture: free-living roaches from a natural lake. How is the welfare of such a system compared to farmed salmonids?

Programme

April 11, 2007

10.00 Opening of the ABILDGAARD/COST/SCOFDA workshop by research school leader Kurt Buchmann, SCOFDA

10.15 GyroDb – a home for gyrodactylids on the web by Andy Shinn, University of Stirling, Stirling, Scotland

11.00 Coffee break

11.30 Welfare aspects of cardiac diseases in farmed salmonids by Trygve Poppe, Norwegian School for Veterinary Sciences, Oslo, Norway

12.15 Lunch break at GIMLE, KVL

13.00 Waging war on white spot: The mechanical device to control *Ichthyophthirius multifiliis* infections in trout farms and improve welfare by Andy Shinn, University of Stirling, Stirling, Scotland

13.30 Breeding and welfare by Hans Magnus Gjøen, Norwegian University of Life Sciences, Oslo, Norway

14.00 Coffee break

15.00 Global aspects of fish nutrition by Anders Kiessling, Norwegian University of Life Sciences, Oslo, Norway

15.45
Breeding for disease resistance in fish by Mark A. Henryon, Hanne Jørgensen & Mette Hansen, University of Aarhus, Denmark

16.00 Vaccination of rainbow trout against yersiniosis – how and when does it work? by Martin K. Raida & Kurt Buchmann, University of Copenhagen, Frederiksberg, Denmark

16.15 Yersiniosis in Atlantic cod by Bjarnheidur K Gudmundsdóttir*, Slavko H. Bambir and Sigrídur Gudmundsdóttir, Institute for Experimental Pathology, University of Iceland, Keldur, Reykjavík, Iceland

16.30 Discussion

17.00 Closing of the first day of the SCOFDA workshop

18.00- 21.00 Dinner at the Falconer Hotel Restaurant, Falkoner Allé 9, 2000 Frederiksberg

April 12, 2007

10.00 Opening of the second day of the COST/SCOFDA workshop

10.15 Functional morphology of the opisthaptoral musculature of *Gyrodactylus* and effects on the host by Andy Shinn & James Bron, University of Stirling, Stirling, Scotland

10.30 Welfare of organically farmed rainbow trout: Legislation and Preliminary experiences by Henrik Rosendahl Kristiansen, Eatguide Research & Consultancy Services, Kolding, Denmark

11.00 Coffee Break

11.30 BEAST: Benedeniid Enumeration And Segmentation Technique. By Andy Shinn[1], Ian Whittington[2] & James Bron[1], Institute of Aquaculture, University of Stirling, Stirling, UK & [2]School of Earth & Environmental Sciences, University of Adelaide, Australia

12.00 Lunch

13.00 Ranavirus infection in Pike *Esox lucius* (L) - Experimental studies of pathogenicity and environmental factors as part of the RANA-project by Britt Bang Jensen & Ellen Ariel, National Veterinary Institute, Danish Technical University, Aarhus, Denmark

13.15 Leukocytes of rainbow trout – can we identify them? By Jacob G. Schmidt & Bent Aasted, University of Copenhagen, Frederiksberg, Denmark

13.30 Gill parasitic diseases in rainbow trout cultured in model trout farms by Isam Saeed, Thomas B. Larsen, Jose Bresciani & Kurt Buchmann University of Copenhagen, Frederiksberg, Denmark

13.45 Temperature dependent expression of immune-relevant genes in rainbow trout following *Yersinia ruckeri* vaccination by Martin K. Raida & Kurt Buchmann, University of Copenhagen, Frederiksberg, Denmark

14.00 Coffee break

14.30 Operational Welfare Indicators (OWI) in Rainbow Trout Farming by Kurt Buchmann, University of Copenhagen, Frederiksberg, Denmark

15.00 Discussion

16.00 Closing of the Abildgaard/COST/SCOFDA workshop

ABSTRACTS

GyroDb: A home for gyrodactylids on the web

Andy Shinn[1], Phil Harris[2], Jo Cable[3], Tor Bakke[4] & James Bron[1]
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[2]School of Education, University of Nottingham, UK
[3]School of Biosciences, Cardiff University, UK
[4]Zoological Museum, University of Oslo, Norway

Basic taxonomic and biological information concerning many of the 400 known species of *Gyrodactylus* is frequently difficult to access, often being published in grey literature or inaccessible journals. Even when such data are available, images and descriptions of the taxonomically informative attachment hooks are of variable quality. This paper describes the development of a web-based resource, "GyroDb", and associated databases to provide access for researchers to current descriptions and data for the known species of *Gyrodactylus*. GyroDb, although still under development, will provide search facilities to locate data for particular *Gyrodactylu*s or host species, a full library of refereed hook images, authored reviews of information relating to selected species and links to available molecular resources. The site will also provide details of parasite distribution and ecology. In addition the site will allow users to submit new data which will be refereed to ensure quality and consistency before upload to the publically accessible databases. Links to relevant sites such as FishBase and researcher and facility homepages will also be provided. Further projected developments include the provision of tools allowing the semi-automated identification of key species. It is intended that this resource will allow a broad range of users to arrive at positive, authoritative, identifications of their own specimens without the need for extensive prior experience in gyrodactylid taxonomy. A provisional version of GyroDb may be accessed at www.GyroDb.net. The concept of 2D and 3D virtual digital holotypes, which might be employed as rapidly accessible proxies for archived counterparts, will also be discussed.

Welfare aspects of cardiac diseases in farmed salmonids.

Trygve T. Poppe
Norwegian School of Veterinary Science
Oslo, Norway

The heart is involved in several infectious diseases in fish and reflects the importance of the organ in disease development and in diagnostic work. In addition, numerous idiopathic cardiac diseases have been identified in farmed salmonids in recent years. The heart has an enormous plasticity and variation and may respond to changes in the environment as well as to physiological changes. Some cardiac diseases are fairly well understood, while others seem to have a diffuse and complex aetiology. Deviations from normal (ideal?) morphology and structure will compromise the function of the heart and may have consequences far wider than those associated directly to the heart. As many of the pathologic conditions associated to the heart appear to be the result of modern, intensive farming practices, they should be regarded as production-related diseases. Such diseases also represent important animal welfare and ethical issues in the interphase between profitability,

fish health and husbandry practices. As such, they should be of concern to fish health personnel serving the fish farming industry. An overview of relevant cardiac diseases in farmed salmonids will be given, and the consequences for the fish will be described. Possible mechanisms and welfare aspects of these conditions will be addressed.

Waging war on white spot: The efficacy of a mechanical device for the control of *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliophora) a commercial rainbow trout, *Oncorhynchus mykiss* (Walbaum), culture systems

Andy Shinn[1], Sara Picon Camacho[1], Bob Bawden[2], Gil Ha Yoon[1] & Nick Taylor[1]
[1]Institute of Aquaculture, University of Stirling, UK
[2]Pisces Engineering Ltd., Easter Poldar, Thornhill, Stirling, UK

Ichthyophthirius multifiliis is recognised as one of the most pathogenic diseases of wild and cultured freshwater fish; infections establishing in hatchery systems proliferate quickly and result in mortalities if left unregulated. To find an environmentally safe, non-chemical alternative to controlling infections, a mechanical device to remove cysts from commercial trout raceways was constructed and tested. The primary mechanical device consisted of a special suction head connected to a pump that was used to vacuum the bottom of hatchery raceways. A secondary and equally necessary device was the use of low-adhesion polymer sheeting that was used to line concrete raceways. Following the testing of a series of raceway linings, the most efficacious product in preventing cyst attachment was used to line three raceways each measuring 6m (length) × 1m (width) × 1m (depth) in a commercial trout hatchery in Scotland. Over a period of three months, each lined raceway was vacuumed once per day; the three control raceways were brushed as normal. Every two weeks, twenty fish from each raceway were sampled and the total number of trophonts on each fish were determined. Statistical analysis confirmed the efficacy of the mechanical device in reducing establishing trophonts numbers by 99.4+% ($p<0.0001$). To ascertain the benefits of the polymer sheeting lining raceways alone in reducing infections, the use of the suction head was withheld and infection levels on fish in raceways were followed for another month. Trophont numbers on the fish in the lined raceways were observed to slowly increase but remained ~39% lower than levels on the control fish.

Breeding and welfare

Hans Magnus Gjøen
Norwegian University of Life Sciences, Oslo, Norway

Domestication is a process that is closely linked with any fish breeding program. This process is propelled by natural selection in the farm environment and through correlated responses for the traits deliberately selected for. Current knowledge in stress physiology and from estimates from experiments suggests that selection for growth rate and low stress could have positive effects in the fish production cycle in the following points:

- Increased quality of the end product
- Increased welfare
- Disease resistance

- Decreased feed waste
- Decreased frequency of premature males

The challenge that needs to be addressed is to do the selection for low stress individuals in an efficient way. Various methods have been suggested and will be discussed in the presentation. Also potential negative side effects of artificial selection on animal welfare will be addressed.

Global aspects of fish nutrition

Anders Kiessling
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An annual growth rate of more than 6% makes fish farming the fastest growing animal production worldwide. Demand for compound feed for fish will exceed 20 mill tons in 2010, and is expected to continue growing. The potential average annual production of fish meal is 6-7 mill tons and of fish oil 1 mill tons (IFFO, 2007). Currently, about half of the fish meal production is used for fish feed, and fish meal has become a limiting resource. This is reflected by an increase in price from approximately 400 US $/ton in 2001 to 1,400 US $/ton in early 2006 (Fig. 1). Prices are expected to stabilise at a level above 1,000 US $/ton (Globefish, 2006). Simultaneously, the price of soybean meal (SBM), which is an indicator of protein price in the commodity market, has fluctuated around 200 US $/ton. Growth in fish farming depends on optimal use of the limited marine protein resources and increased use of proteins from other, sustainable sources. This shortage of fish based feed sources has forced feed sources for carnivorous fish in to a transition phase from being fish-based to multi-source-based. In the near future, only plant sources offer a viable alternative to fish meal. Highly and properly processed plant protein sources, such as soy or potato protein concentrates or wheat gluten, can replace fish meal in diets for carnivorous fish in a way that can support rapid growth and high feed efficiency. These plant protein sources, however, expensive due to the cost of wet treatment and subsequent drying. The drawback with plant sources as animal feed, in a longer perspective, is their suitability as human food. Therefore, an increased use of sources not in direct competition with human food is the only sustainable alternative. *By-products from human activities, used directly or via fermentation by micro-organisms*, may offer such sustainable alternatives as feed sources for carnivorous fish. The huge advantage of this later source is naturally their potential as key step in a sustainable society, recirculation human waste or by-products from bio-energy. The advantage of fish compared to other farmed animals in such recirculation from waste back to human food is several folds. Firstly fish in contrast to ruminants but also to a lesser degree to pig and poultry digest and deposit protein without releasing methane, a highly climate driving gas. Secondly fish is poikilotherms, i.e. do not as e.g. a pig or poultry spend 40% of its nutrients on heat increment. Thirdly fish is without competition the animal demanding less resource for reproduction and the parental generation. Finally, but not least, fish has enzyme systems capable of metabolising the extremely high content of nucleotides that characterise micro-organisms and turn it in to non harmful and water soluble substances.

Breeding rainbow trout for resistance to disease

Mette HH Hansen, Hanne Jørgensen & Mark Henryon
University of Aarhus, Faculty of Agricultural Sciences, Foulum,, Denmark

We are searching for traits that indicate disease resistance in rainbow trout, enabling us to breed for improved resistance. We have previously shown that we can breed rainbow trout for resistance to the diseases, enteric redmouth disease, rainbow trout fry syndrome, and viral haemorrhagic septicaemia. We demonstrated that resistance to each of these diseases was heritable, and genetic correlations among the resistances were weak. These findings prompted us to begin two projects that: (a) explore the underlying (genetic) mechanisms that confer resistance, and (b) identify traits that indicate resistance. In the first project, we are identifying genes that are expressed differentially in high and low resistance trout following infection with viral haemorrhagic septicaemia using microarrays and quantitative PCR. In the second project, we are identifying functional variation in MHC peptide-binding regions for resistance to enteric red-mouth disease, rainbow trout fry mortality syndrome, and viral haemorrhagic septicaemia. We will correlate this functional variation with quantitative genetic estimates, to see how MHC genotypes may contribute to breeding strategies. We expect that the project assessing gene expression will highlight underlying immunological pathways that provide trout with resistance to viral haemorrhagic septicaemia, and help us to identify candidate genes that confer resistance in rainbow trout. The project involving functional variation in MHC peptide-binding regions will identify MHC haplotypes that infer resistance to enteric red-mouth disease, rainbow trout fry mortality syndrome, and viral haemorrhagic septicaemia.

Bath vaccination of rainbow trout against yersiniosis – when and how does it work?

Martin K. Raida & Kurt Buchmann
University of Copenhagen, Frederiksberg, Denmark

Studies have been conducted on the temperature-dependent effect of bath vaccination of rainbow trout against *Yersinia ruckeri* O1. Protection of rainbow trout fry against challenge, following bath vaccination with a bacterin of *Yersinia ruckeri* O1, the bacterial pathogen causing enteric red mouth disease (ERM), was investigated at 5, 15 and 25° C. Rainbow trout fry were kept at controlled temperatures for two month before they were immersed in a commercial *Yersinia ruckeri* O1 bacterin for 10 minutes. Control groups were sham vaccinated using pure water. Fish were challenged with *Yersinia ruckeri* O1 one and two month post vaccination at the three temperatures. Protection of vaccinated fish was seen one and two month post vaccination in rainbow trout reared at 15° C. There was no effect of vaccination in rainbow trout reared at 5 and 25° C. Spleen tissue was sampled from 5 vaccinated and 5 control fish at each rearing temperature: 0, 8, 24 and 72 hours post vaccination in order to analyse immune gene transcript profiles with real-time RT-PCR (qRT-PCR). Complement factor C3 and C5 were down regulated in vaccinated fish kept at 5 and 15° C, relative to unhandled controls. Expression of the pro-inflammatory cytokines IL-1β, INF-γ and TNF-α, and the acute phase protein precerebellin, were up-regulated at all samplings post vaccination in fish reared at 25° C. The study has shown that bath vaccination offers protection at 15° C and not at 5 and 25° C. The implications for practical fish farming will be discussed.

Yersiniosis in Atlantic cod

Bjarnheidur K Gudmundsdóttir*, Slavko H. Bambir and Sigrídur Gudmundsdóttir
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Yersiniosis is a bacterial disease of various fishes, including salmonids, eels, goldfish, sole, sturgeon and turbot, caused by the Gram negative bacterium *Yersinia ruckeri.* Acute infections in salmonids are also referred to as enteric red mouth (ERM). The bacterium has been isolated from fish throughout Europe, North and South America, Australia and New Zealand. In this study a disease outbreak is reported concerning yersiniosis in farmed Atlantic cod. The cod with an average weight of 35g were cultivated in an experimental fish farm, which also cultivates other marine fish species, i.e. halibut, turbot, European wolffish and spotted wolffish. A fish farm, which has a history of *Y. ruckeri* infections in Artic charr, is located within 1000 m from the marine fish farm. Inspections of the cod population for one week, following the first isolation of *Y. ruckeri,* revealed a mortality of 1% . *Y. ruckeri* was confirmed in kidney samples from all dead fish by standard bacteriological methods. Gross pathological alterations involved exophthalmia and haemorrhages in the eyes, petecchial haemorrhages on the belly, fins, at fin bases and tail, and diffuse haemorrhages on the head. Histopathological analysis revealed bacterial colonies and pathological changes in many organs, including heart, brain, spleen, liver, and intestine.

Functional morphology of the opisthaptoral musculature of *Gyrodactylus* (Monogenea)

Andy Shinn & James Bron
Institute of Aquaculture, University of Stirling, Stirling, UK.

According to current models of attachment in *Gyrodactylus* spp., marginal hooks at the outside edges of the opisthaptor provide the principal force of attachment whilst large central hooks, hamuli, provide a system preventing accidental dislodgement and assist the action of the marginal hooks. In this model, attachment is achieved by the alternating action of two systems of muscles attached respectively to the hamuli and to the marginal hooks. These models have been principally based upon observations of the morphology and disposition of the skeletal elements, with the function and identity of attendant musculature and suspensory elements being largely theoretical rather than derived from direct observation. In current models, relaxation or contraction of the muscles connected to the hamuli manoeuvres them over the extremities of the ventral bar and allows them to pivot, effectively raising or lowering the opisthaptoral dome. Under reduced opisthaptoral tension, the independent gaffing activity of the marginal hooks is able to ensure a secure attachment to the host's epidermis. Repositioning of the hamuli then raises the opisthaptoral dome to tension the peripheral marginal hooks. The sequence of attachment is complete when all the muscles associated with the hooks are in a state of relaxation but held securely and under tension by the surrounding, stretched opisthaptoral dome. The current study investigates the role of these and associated muscles in the attachment process as determined from studies using SEM, TEM and confocal laser microscopy.

Welfare of organically farmed rainbow trout: Legislation and Preliminary experiences

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The increasing demand for organically farmed food is driven by increasing awareness that "we are what we eat". The fear of global epidemic diseases such as bird flu and mad cows disease has a dramatic influence on national disease control and consumers behaviour. Local food scandals such as dioxins in chicken feed, discovery of bad meat on the market, pathogenic bacteria in drinking water and viral diseases in food has tightened up the food control and changed the shopping pattern. Moreover, the revelations of mistreated farmed animals and bad influence of farming on the environment, has caused that the parliaments passed the legal basis for marking of food such as the organic Ø mark. Organically farmed vegetables, eggs, milk and other diary and meat products have been accepted on the market, but fish lacks behind. Recently the Danish Parliament passed the two regulations behind organic fish farming: 1) Regulation No. 114 of 23/02 about organic fish farming and 2) Regulation No. 115 of 23/02 about foodstuffs in organic fish farming. This speech provide an overview of the differences between conventional and organic farming of rainbow trout in Denmark. Preventive measures against diseases will be explained because the regulations allow medication only once during the on-growing phase. The fish farmer must therefore include multiple factors in the management strategy such as: a) disease resistance of the broodstock, b) the stocking density, c) oxygen level in the ponds, d) feeding strategy especially during the summer and e) prevention of stress due to physical and chemical parameters. Interesting on going research in stress monitoring will be referred to. The audience will see slides from the experimental Skravad Mill Fish farm and listen to preliminary practical experiences. This includes a preliminary judgement of the quality of the fish and the meat, which has been used to develop a couple of recipes. The recipes will be used to guide the consumers purchasing the organically farmed trout in a local fish monger, who participate in the "eat fish twice a week campaign".

BEAST: Benedeniid Enumeration And Segmentation Technique.

Andy Shinn[1], Ian Whittington[2] & James Bron[1]
[1]Institute of Aquaculture, University of Stirling, Stirling, UK.
[2]School of Earth & Environmental Sciences, University of Adelaide, Australia

Infections of *Benedenia seriolae* (Monogenea) on sea cage-cultured stocks of yellowtail kingfish *Seriola lalandi* and *Neobenedenia melleni* on marine farmed barramundi *Lates calcarifer* are a major concern for fish health and welfare. Heavy infections, if left untreated, can cause epithelial hyperplasia, haemorrhage and may lead to secondary infection and osmotic stress resulting in host mortality. In order to manage these pathogens effectively and to allow strategic deployment of chemotherapeutic controls, frequent monitoring of parasite prevalence, incidence and life-cycle

stages is required. Currently, such monitoring programmes have high manpower / economic costs. The BEAST (Benedeniid Enumeration and Segmentation Techniques) software we have developed allows for the rapid semi-automated quantification of benedeniids and other large monogeneans (261 specimens in ~90s), using low cost imaging hardware and a standard low cost PC. The programme allows users to perform a range of operations on a single image field comprising a sample or sub-sample of monogeneans removed from a single or multiple hosts. The semi-automatic operations include counting individuals and measuring a range of morphometric parameters and shape descriptors which can be used to separate *Benedenia* specimens from those of *Zeuxapta seriolae* which occur on the gills, the data from which can be used to inform farm management and control strategies.

Ranavirus infection in Pike *Esox lucius* (L) - Experimental studies of pathogenicity and environmental factors as part of the RANA-project.

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

Systemic ranaviruses have been associated with the decline of amphibians and both wild and farmed freshwater fish species in Australia, Asia, North America and Europe. Several virus isolates associated with mortalities have been found, posing a possible threat to the aquaculture industry. It is speculated that amphibians may act as vectors and propagate the spread of ranaviruses between separate fish habitats. Therefore, the EU WP6-project “RANA” (Risk assesment of new and emerging systemic iridoviral diseases for European fish and aquatic ecosystems) was initiated in June 2005.The project involves partners in five EU-countries, NVI-DTU being the Danish participant. One of the main challenges is to identify susceptible species and investigate the pathogenesis of the virus infections. In this study, Pike (*Esox lucius*) were subjected to infection trials with six different ranavirus isolates, in order to study the pathogenicity of these isolates for a common European wild-living freshwater fish species. The ranaviruses have been isolated from Pikeperch (*Stizostedion lucioperca*), European Catfish (*Ictalurus melas*), European Sheatfish (*Siluris glanis*), Redfin Perch (*Perca fluviatilis*), Shortfin Eel (*Anguilla australis*) and Leopard frog (*Rana pipiens*). Pike fry of 0.1 grams were exposed to the virus by immersion at two different temperatures, consistent with natural temperature ranges in European freshwater. All the viruses, except for the amphibian isolate were pathogenic to the pike fry, inducing mortalities of 93-98% in warm water and 51-100% in cold (compared to 84% and 37% in the negative control groups for warm and cold water, respectively). In total, 31% of the samples showed positive CPE when cultured on EPC-cells. It was investigated what influence the number of fish in sample, virus isolate, days in trial and temperature had on a possible positive CPE. Immunohistochemistry and immunoflourescence were performed, using a polyclonal antibody specific for one of the virus isolates. The findings indicate that systemic ranaviruses from different fish species are highly pathogenic to pike fry and the virus can spread easily from one fish species to another.

Leukocytes of rainbow trout – can we identify them?

Jacob G. Schmidt & Bent Aasted
University of Copenhagen, Frederiksberg, Denmark

In humans, monoclonal antibodies (MAbs) are important tools in the isolation and study of cells of the immune system; the leukocytes. MAbs are potentially equally important in studies of piscine leukocytes, but such investigations are hampered by a lack of MAbs. In an attempt to improve on this situation, a panel of MAbs was tested by flow cytometry and immunoprecipitation studies for reactivity with leukocytes from the blood and head kidney of rainbow trout, *Oncorhynchus mykiss*. Of 28 MAbs, seven had already been described, and were included as positive control references. Nine were negative in flow cytometry. Of the remaining twelve positive, uncharacterized MAbs, none likely supplemented the already published MAbs regarding cell specificities: Two labelled thrombocytes; three labelled thrombocytes and neutrophils (and possibly monocytes/macrophages); three labelled almost all leukocytes, although thrombocytes were only weakly labelled; two labelled all leukocytes except thrombocytes; and two labelled some lymphocytes and a population of unidentified cells with high forward scatter and low side scatter properties. A total of 13 MAbs successfully immunoprecipitated antigen, three MAbs gave uncertain results, and ten were negative. Two MAbs were not used in the immunoprecipitation studies. In the presentation, emphasis will be placed on the thrombocyte-specific MAb 15 and the two MAbs N2 and N3, of which the former is specific for Ig light chain. The monoclonal antibodies were kindly provided by Uwe Fischer and Bernd Köllner, FLI, Insel Riems, Germany and Karsten Skjødt, SDU, Odense, Denmark.

Gill parasitic diseases in rainbow trout cultured in model trout farms

Isam Saeed, Thomas B. Larsen, Jose Bresciani and Kurt Buchmann
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During a parasitological survey conducted from January 2006 to January 2007 a number of parasites were encountered in rainbow trout *Oncorhynchus mykiss* gills cultured in modern recirculating systems. The pathogens include the skin flagellate *Icthyobodo necator* and the skin ciliates *Ichthyophthirius multifiliis, Trichodina nigra, T. mutabilis, T. fultoni, Chilodonella* sp., *Apiosoma* sp., *Epistylis* sp., and *Ambiphrya* sp..Different methodologies were applied including light microscopy (7-1000 X magnification), scanning electron microscopy and histology. Materials investigated comprised fresh mucus smears, whole mounts of gill filaments and formalin fixed gill arches. The results will be discussed in relation to management practice and farm environment.

Temperature-dependent expression of immune-relevant genes in rainbow trout following *Yersinia ruckeri* i.p. vaccination

Martin K. Raida & Kurt Buchmann
University of Copenhagen, Frederiksberg, Denmark

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

Operational Welfare Indicators (OWI) in Rainbow Trout Farming

Kurt Buchmann
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Rainbow trout (*Oncorhynchus mykiss*) is an anadromous salmonid which originates from North America but has been transplanted to all continents where the climate allows cultivation of this species. Due to the extensive knowledge on the life cycle, ecology, physiology, nutrition, behaviour, disease control and marketability of this teleost it has become one of the most popular species in cold-water aquaculture. The latest production estimates indicate that the world annual production is around ,000 metric tonnes. The species is reared both under freshwater and marine conditions and a range of techniques and rearing systems are being used for propagation, rearing and on-growing. The main producers in Europe comprise Norway, Denmark, Sweden, Finland, Poland, Germany, Turkey, Italy, Spain and France.

Welfare aspects of rainbow trout farming

When teleosts are domesticated and introduced into artificial systems a number of conflicts between the demands of the species in question and the technology and economy of the husbandry systems may arise. In order to address these questions on a broader European scale the COST Action 867 was established in 2006 following an initiative of Dr. Anders Kiessling, University of Life Science, Oslo, Norway. Scientists from most European countries are taking part in the action which eventually could lead to important guidelines for future fish farming. Thus, under this umbrella a working group on Operational Welfare Indicators (WG2) has been set in action in order to develop tools for future management of rainbow trout welfare. A website has been established www.welfare.com and a COST 867 meeting has been planned in Varese Italy, May 2007.

Operational Welfare Indicators OWI

These indicators are defined as ***operational*** when they can be corrected instantly whereas they are designated ***strategic*** when they can not be corrected instantly. Both types of indicators are referred to as ***direct*** if they are related to the fish and as ***indirect*** if they refer to the system. When addressing operational welfare indicators in rainbow trout production it is important to focus on freshwater and marine systems separately. Further, production of fry and fingerlings are different from on-growing of larger fish and the technologies applied are so different that general discussions and conclusions may be difficult. Therefore it is necessary to consider the different rearing systems such as flow-through, recirculation, concrete raceways, fibre-glass tanks, earth ponds and net-pen-systems both in fresh and salt water. For an initial analysis of these parameters it has been suggested to focus on the following parameters: OWI: Fish farm conditions, OWI: Water quality, OWI: Nutrition, OWI: Behaviour and appearance of fish, OWI: Disease signs, OWI: serological/physiological conditions, OWI: Transport conditions, OWI: Slaughtering.

Practical application of OWI

When entering the rainbow trout farm (freshwater, marine, flow-through, recirculated, fry, fingerlings, on-growers, transport, slaughter) a series of inspections and observations should be taken in order to determine the state of welfare on the farm (Table 1). These should refer to the fish but also the system (fish density, unit size), fish appearance (size, condition factor, skin, fin, eye and gill integrity and colour), fish behaviour (location in water column, air-gasping, balance, activity), water quality (oxygen, ammonia, BOD) (Table 2), feed quality (essential nutrients, oxidation state of lipids, contaminants) (Table 3) and to clinical signs (additional abnormalities). Health inspections should be conducted and include virology, bacteriology and parasitology (Table 4). Serological parameters may be included if possible due to the fact that factors such as serum cortisol, glucose, lactic acid and others may reveal both acute and chronical welfare problems. Thus antibody titers may reveal previous or present infections against specific pathogens.

Operational OWI: Indirect OWI related to the system

When observing excessive densities of fish in a system this may be instantly corrected. Likewise, poor water quality conditions may be improved instantly.

Operational OWI: Direct OWI related to the fish

Basic health inspections on the farm site may reveal that clinical signs of the fish could be caused by excessive abundance of ectoparasites/ectocommensals on the gills, fins and skin of the rainbow trout. The problems associated with this may be corrected instantly through water treatment using formaldehyde, hydrogen peroxide, sodium percarbonate, sodium chloride or other disinfectants. If no other infections or farm conditions are interfering an instant improvement of the fish and its behaviour may be observed following such treatment.

Strategic OWI: Indirect OWI related to the system

The physico-chemical conditions in the production plant can in some cases be corrected. Thus, in recirculated fish farm systems operating limited water volumes it is possible to replenish fish tank water (adjusting BOD, salinitiy), re-oxygenate water, adjust salinity by adding sodium chloride or to adjust pH by adding hydrochloric acid or sodium hydroxide. In larger enterprises such as net-pen culture in the sea it is not always feasible to correct the problems instantly.

Strategic OWI: Direct OWI related to the fish
The issue of health conditions may prove to be a good example of strategic OWI. If the health problem of the fish is not related to superficial colonisation with ectocommensals or parasites which can easily be managed as described above it is necessary to make a plan for correction of the adverse conditions. This may include stamping out (if the disease is not treatable), drug treatment (if available), radical change of water source or feed source.

How can fish farmers, farm inspectors and authorities use OWI?
The systematic observations of important farm and fish parameters may be powerful tools for future description of the welfare systems in a particular farm environment. However, due to the extreme variation between farming systems the specific farm type should be addressed in all cases.

Table 1. Initial observation of parameters related directly to the fish in the farms and the possibility for instant correction

Observation	Instant correction possible
Mortality	+/-
Condition factor	-
Epaxial musculature	-
Hypaxial musculature	-
Exophthalmia	-
Colour/pigmentation	-
Skin	-
Fins	-
Gills	-
Ulcers	-
Balance disturbances	+/-
Air gasping	+/-
Scratching	-
Lethargy	+/-
Visible parasites	+/-

Table 2. Water quality parameters and their possible use as operational welfare indicators

Parameter	Instant correction possible
Temperature	+/-
Salinity	+/-
pH	+/-
Water flow	+/-
Turbidity	+
BOD	+
Ammonia	+
Nitrite	+
Nitrate	+
Phosphate	+

Table 3. Nutritional parameters and their possible use for OWI

Nutritional element	Instant correction possible
Proteins	
Amino acids	+
Lipids	
Fatty acids	+
Carbohydrates	+
Vitamins	+
Minerals	+
Antoxidants	+
Immunostimulants	+
Contaminants	+/-

Table 4. Some infections with various pathogens in rainbow trout farming (freshwater/marine) and relevance as operational welfare indicators. Possibility for control indicated and if this correction can be performed instantly

Pathogen	Control method available	Instant correction possible	Instant correction not possible
Virus			
VHSV	+	-	+
IHNV	+	-	+
IPNV	+	-	+
Bacteria			
Flavobacterium psychrophilum	+	-	+
Yersinia ruckeri	+	-	+
Aeromonas salmonicida	+	-	+
Vibrio anguillarum	+	-	+
Parasites			
Ectocommensals			
Apiosoma	+	+	-
Ambiphrya	+	+	-
Capriniana	+	+	-
Epistylis	+	+	-
Ectoparasites			
Trichodina	+	+	-
Chilodonella	+	+	-
Skin parasites			
Ichthyobodo	+	+	-
Tetrahymena	+	-	+
Ichthyophthirius	+	-	+
Gyrodactylus	+	+	-
Argulus	+	-	+
Lepeophtheirus	+	-	+
Caligus	+	-	+
Intestinal parasites			
Spironucleus	+	-	+
Eubothrium	+	-	+
Crepidostomum	+	-	+
Eye parasites			
Diplostomum	+	-	+
Tylodelphys	+	-	+
Kidney parasites			
Tetracapsuloides bryosalmonae	+	-	+
Cartilage parasites			
Myxobolus cerebralis	+	-	+

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