DAFINET WORKSHOP

IN COLLABORATION WITH BANGFISH AND PARAFISHCONTROL

SUSTAINABLE FISH HEALTH CONTROL

OCTOBER 23RD, 2018

Program and book of abstracts

Venue:

Grand lecture theatre , 1-01

Bülowsvej 17

University of Copenhagen

1870 Frederiksberg C

Denmark

Organized by:

DAFINET, www.dafinet.dk

BangFish, Danida

ParaFishControl, Horizon 2020

Book of abstracts

DAFINET, BangFish and ProFish Workshop
October 2018
University of Copenhagen, Denmark

Book of abstracts edited by
Per W. Kania and Kurt Buchmann

Photo and illustration by
Kurt Buchmann, Copenhagen, Denmark

Printed by
Frederiksberg Bogtrykkeri 2018
Frederiksberg, Denmark

DAFINET WORKSHOP

SUSTAINABLE FISH HEALTH CONTROL

OCTOBER 23RD, 2018

Venue:

Grand lecture theatre, Auditorium 1-01, Bülowsvej 17

University of Copenhagen, 1870 Frederiksberg C, Denmark

Program - Tuesday October 23rd, 2017

09:00 Welcome address by DAFINET leader Kurt Buchmann

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

09:15 Sonal Patel

Institute of Marine Research, Bergen, Norway

Microbiome of salmon: host-microbial relationships

09:45 Jakob Skov

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Effects of soluble immunostimulants on mucosal immune responses in rainbow trout immersion vaccinated against *Yersinia ruckeri*

10:00 Maria Sokolova

National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), Denmark

Spatial patterns in infection of cod *Gadus morhua* with the seal-associated liver worm *Contracaecum osculatum sensu stricto* from the Skagerrak to the central Baltic Sea

10:15 Agung Cahyo Setyawan

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Endoparasitic helminths in Baltic salmon *Salmo salar* L.: Ecological implications

10:30 Sazedul Hoque

Faculty of Fisheries, Patuakhali Science and Technology University, Bangladesh

Yellow fillets of *Pangasius* in Bangladesh: Prospects and Challenges in International Market

10:45 Jiwan Kumar Chettri

National Veterinary Institute, Technical University of Denmark, Denmark

Experimental anal infection of rainbow trout with *Flavobacterium psychrophilum*: A novel challenge model

11:00 Niels Lorenzen

National Institute of Aquatic Resources, Danish Technical University (DTU Aqua), Denmark

Use of recombinant VHS virus encoding GFP for functional studies of anti-viral immunity in rainbow trout

11:30 Lunch at LAP, IVH, Thorvaldsensvej 57, Frederiksberg – 5 min walk from the venue

13:00 Morten Tønsberg Limborg

Section for EvoGenomics, Natural History Museum of Denmark, University of Copenhagen, Denmark

Applied Hologenomics: Integrating host-microbiota interactions in fish health studies

13:30 Azmi Al-Jubury

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Cercarial dermatitis in Danish freshwater lakes during summer 2018

13:45 Mohammad Lokman Ali

Patuakhali Science and Technology University, Bangladesh

Effect of salinity on the survival and growth of *Pangasius* catfish in southern in Southern Bangladesh

14:00 Louise von Gersdorff Jørgensen

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Associations between the skin parasite *Ichthyophthirius multifiliis* and the immune system of the fish host *Danio rerio*

14:30 Khairul Syahputra

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Association between stress induced by *Ichthyophthirius multifiliis* infection and differential immunological responses in skin, gills, and spleen of rainbow trout, *Oncorhynchus mykiss* (Walbaum)

Coffee and tea break at LAP, IVH, Thorvaldsensvej 57, Frederiksberg – 5 min walk

15:15 Emranul Ahsan

Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh

Detection of bioavailable tetracycline from pangas and tilapia aquaculture production system in Bangladesh using whole-cell biosensor bacteria

15:30 Huria Marnis

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Transcriptomic profiling of Baltic cod (*Gadus morhua*) liver in response to *Contracaecum osculatum* infection

15:45 Seikh Razibul Islam

Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Factors responsible for the off-flavour and yellow colour in *Pangasianodon hypophthalmus*

16:00 Muhammad Abdur Razzak

Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh

Detection of bioavailable Arsenic from pangas and tilapia farms in Bangladesh fed with groundwater by using bioluminescent biosensor bacteria (*E. coli* K12 pJAMA arsR)

16:15 Shaozhi Zuo

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Ecological and molecular links between *Contracaecum osculatum* (Nematoda, Anisakidae) in Baltic cod (*Gadus morhua*) and grey seal (*Halichoerus grypus*)

16:30 Md. Rushna Alam

Department of Aquaculture, Faculty of Fisheries, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh

Effect of hydrogen peroxide, polycyclic musks and salinity on aquatic organisms

16:45 Wrap up by DAFINET leader

18:00 Dinner at Akropolis, H.C. Ørstedsvej 70, Frederiksberg, 15 min walk from the venue

ABSTRACTS

Microbiome of salmon: host-microbial relationships

I. Salinas[1], R. Brown[1], L. Moore[2,3], I.U. Fiksdal[2], Ø. Vågnes[4], H.L. Thim[4], T. Galloway[5], J. Seilo Torgersen[5], S Patel[2,4,]

[1]University of New Mexico, USA
[2]Institute of Marine Research, Bergen, Norway
[3]Department of Biological Sciences, University of Bergen, Norway
[4]Vaxxinova Norway AS, Bergen, Norway
[5]Aquagen AS, Trondheim, Norway

Presenting author: Sonal Patel, sonal.patel@vaxxinova.no

The mucosal surfaces and associated microbiota of fish are an important primary barrier and play an important role in the first line of defense against potential pathogens. Skin and gills are exposed to the surrounding environment and thus analysis of the microbes on the skin and gills may provide useful insights into the dynamics of fish host–microbial relationships. Such analyses may also reveal underlying changes in health status and provide information on factors that affect the composition of the microbiome. Mortalities associated with viral infections such as salmonid alphavirus, lice infestations, and wound-related mortalities are major challenges in Atlantic salmon industry and could be addressed using microbial analyses.

We have studied the microbial composition and the changes in this composition during two experimental SAV infections in salmon. In one study, salmon were exposed to two different doses of SAV and microbiome analysis of skin was compared between non-infected and the infected groups. The results show that there was a shift in the microbial composition upon SAV infection. In the second study, triploid and diploid salmon were exposed to SAV and the microbiome analysis of both gills and skin was carried out and compared with the non-infected groups at time points prior to infection and three weeks post-infection. Prior to experimental SAV infection, the pathogen *Candidatus Branchiomonas* accounted for 7% and 52% of microbial diversity in triploid and diploid salmon, respectively. This taxon was expanded in triploid and diploid three-week controls, as well as the experimental group, effectively wiping out beneficial commensals such as *Oleispira sp.* Preliminary analysis of the skin microbiota shows no significant differences between triploid and diploid salmon under normal conditions prior to infection, while the post-infection samples are being processed.

Wound related mortality is another challenge in salmon aquaculture. Following the mandatory regulations for the control of salmon lice exposes salmon in sea cages to varying lice treatments including chemicals, thermal or mechanical treatments. These delousing treatments damage the epithelial/mucosal surfaces of the skin and aggravates wound-related challenges. Apart from mechanical and lice related wounds, farmed salmon also develop winter-ulcers when infected with the bacterium *Moritella viscos*a. At temperatures below 10 °C this represents a serious fish welfare issue. In the Robust skin project, one of the aims is to characterize the impact of delousing treatments by analyzing the skin microbiome before and after the treatment. The health status before treatment, including epithelial integrity is important and the samples collected from several sea cages before and at two time-points after the hydrolyzer treatment of salmon will help in our understanding of some of the challenges.

Effects of soluble immunostimulants on mucosal immune responses in rainbow trout immersion vaccinated against *Yersinia ruckeri*

J Skov[1], J.K. Chettri [2], R.M. Jaafar [1], P.W. Kania [1], I. Dalsgaard [2], K. Buchmann [1]

[1]*Department of Veterinary and Animal Science, University of Copenhagen, Denmark*
[2]*National Veterinary Institute, Technical University of Denmark, Kgs. Lyngby, Denmark*

Presenting author: Jakob Skov, jask@sund.ku.dk

Immersion vaccination of rainbow trout against *Yersinia ruckeri* infection is an established method to prevent enteric red mouth disease ERM but the effect is inferior to injection vaccination and the duration of protection is limited to less than six months. Adjuvants in vaccines may in general elevate the immune response and the present work elucidates how ERM immersion vaccination of trout in combination with exposure to soluble adjuvants, Montanide™ IMS 1312 VG PR and β-glucan, affects immune reactions. The former adjuvant, when used alone, induced a slightly increased protection (not statistically significant) whereas β-glucan did not increase protection. Adjuvant treated and non-exposed groups showed elevated plasma lysozyme activity after challenge with *Y. ruckeri*. Specific antibody production was not positively affected by combining adjuvant and vaccine. Overall expression of immune genes tested was generally manifold higher in gills compared to skin. Only genes encoding SAA and IL-17c1 were expressed at a higher level in skin. Dynamic differences between the gill and skin compartments were also recorded for genes encoding cytokines (TNF-α, IL-1β, IL-6, IL-10, IL-12, IL-17A/F2, IL-17c1, IL-17c2, IL-22), immunoglobulins (IgM, IgD, IgT), cell markers (CD8α, TCRβ) and acute phase reactants (SAA, lysozyme). These genes were up-regulated 24 h post-vaccination in fish gills exposed to both vaccine-adjuvant combinations when compared to fish exposed to vaccine alone. The reactions decreased after a few weeks and after challenge with bacteria mainly unvaccinated fish responded again. Adjuvants used in combination with immersion vaccine clearly influences immune reactions and may improve duration and protection but further potency tests should be performed.

Spatial patterns in infection of cod *Gadus morhua* with the seal-associated liver worm *Contracaecum osculatum sensu stricto* from the Skagerrak to the central Baltic Sea

M. Sokolova[1], K. Buchmann[2], B. Huwer[1], P.W. Kania[2], U.Krumme[3], A. Galatius[4], J. Hemmer-Hansen[5], J.W. Behrens[1]

[1]National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), Denmark
[2]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
[3]Thünen Institute of Baltic Sea Fisheries, Rostock, 18069, Germany
[4]Department of Bioscience, Aarhus University, Roskilde, 4000, Denmark
[5]National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), Silkeborg, 8600, Denmark

Presenting author: Maria Sokolova, mariaasokolova@yandex.ru

Fish serve as transport hosts to a range of parasites, with potential negative effects on fish health. The ultimate consequences of a specific parasite on the health of fish stocks are however often difficult to assess. In the Baltic Sea, the grey seal *Halichoerus grypus* (Fabricius) population has increased markedly since the early 2000s. *H. grypus* is the main final host to the liver worm *Contracaecum osculatum* (Rudolphi, 1802), a parasitic nematode to which cod *Gadus morhua* (Linnaeus) is one of several transport hosts. Recent investigations have shown a marked increase in prevalence and abundance of infection of this parasite in livers of *G. morhua* inhabiting the central Baltic Sea. Yet no recent knowledge exists on levels of *C. osculatum* infection in *G. morhua* in adjacent areas.

We investigated spatial differences in prevalence and abundance of this parasitic nematode in livers of *G. morhua*, covering a transect consisting of nine areas from the Skagerrak to the eastern part of the central Baltic Sea. We further provide survey data of local abundances of *H. grypus* and harbor seal *Phoca vitulina* (Linnaeus) throughout this transect. Prevalence and abundance of *C. osculatum sensu stricto* in *G. morhua* livers differed significantly between east and west, with highest levels of infection occurring in the low-salinity central Baltic areas. Highly infected fish in the east had significantly lower condition factor than their westerly, less infected conspecifics. Spatial differences in local seal abundance and seal species, salinity and feeding ecology may explain the observed differences in *C. osculatum* infection between eastern and western *G. morhua*.

Endoparasitic helminths in Baltic salmon *Salmo salar* L.: Ecological implications

A.C. Setyawan, S. Zuo, P.W. Kania, K. Buchmann

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Presenting author: Agung Cahyo Setyawan, agung@sund.ku.dk

Parasites in fish serve as ecological indicators as they reflect migration routes, feeding behavior and immune status of the host and thereby represent valuable biological information. We have performed a parasitological investigation of sea-running Baltic salmon caught in the Southern Baltic Sea in order to illustrate the unique biology of this fish stock. It represents a strain of the Atlantic salmon *Salmo salar*, which for several millennia, since the latest glacial period, has been isolated in the semi-enclosed brackish Baltic Sea with limited out- and inward migration to and from the North Sea. A total of 24 specimens of salmon (total body weight ranging from 4.2 to 14.2 kg, total body length 80-105 cm) were caught by spoon-bait during their feeding migrations in the southern Baltic sea near the island Bornholm at April 27, 2017. Fish were necropsied few hours after catch and internal organs (esophagus, stomach, pyloric caeca, intestine, liver, spleen) were removed and frozen immediately at minus 20°C. Following thawing the material was subjected to a parasitological investigation focusing on endoparasitic helminths. The stomach contained a high number of the hemiurid digeneans *Brachyphallus crenatus* and *Hemiurus luehei* (96% and 63% prevalence, respectively) reflecting previous feeding on clupeids. The pyloric region was heavily parasitized by the cestode *Eubothrium crassum* (100% prevalence), reflecting feeding on smaller pelagic fishes. The intestine contained *Schistocephalus solidus* liberated from infected sticklebacks representing an important food source in the area (17% prevalence). The acanthocephalan *Echinorhynchus truttae* (prevalence 54%) was found in the intestine together with the adult nematode *Hysterothylacium aduncum* (29% prevalence). The liver was parasitized by third stage nematode larvae of *Hysterothylacium aduncum* and *Contracaecum osculatum* but these larvae were growth stunted and encapsulated. Positive correlations between host size and infections with nematode larvae and stomach trematodes indicated an increasing share of clupeids (transport hosts) in food with increasing size. Negative correlation between size and acanthocephalan infection may reflect decreased feeding on benthic crustaceans (intermediate hosts) with increasing host size. Stomach content analysis reflected a mainly fish based diet (stickleback, herring, sprat, garfish, cod). The results are discussed in light of the ecology of the Baltic Sea as the parasite fauna differs markedly from North Atlantic waters.

Yellow fillets of Pangasius in Bangladesh: Prospects and Challenges in International Market

S. Hoque[1], M.H. Haque[2], I. Hossain[2], S. Mahmud[1], M.A. Razzak[1], A.K. Mandal[3], Badiuzzaman[3], T. Rahman[3], M. Frederiksen[4], E.P. Larsen[5] and M. Nielsen[6]

[1]Faculty of Fisheries, Patuakhali Science and Technology University, Bangladesh.
[2]Faculty of Fisheries, Bangladesh Agricultural University, Bangladesh.
[3]Faculty of Business Administration and Management, Patuakhali Science and Technology University, Bangladesh.
[4]Senior Project Manager, Eurofish International Organisation, Denmark
[5]Senior Advisory Scientist, National Institute of Aquatic Resources, Technical University of Denmark, Denmark.
[6] Associate Professor, Food Resource and Economics, University of Copenhagen, Denmark.

Presenting Author: Sazedul Hoque, sazedul.fst@pstu.ac.bd

Farming of pangasius (*Pangasianodon hypophthalmus*) have grown significantly over the last two decades with Vietnam being largely the only country contributing to export. Bangladesh is worldwide the third largest producer, but despite of that export is almost non-existing.

The purpose of this article is to assess why fillets of pangasius are yellow miscolored in Bangladesh and to analyse how solving the problem can lead to export.

The study revealed that farming practices (high fish stocking density, infrequent water exchange facilities) and low water quality parameter (low dissolved oxygen and high ammonia) of the farm are imperative for yellow fillet of pangasius. Artificial and natural pigments (carotenoids and astaxanthin), nutrient compositions in feed, chromatophore cells, post-harvest handling of fish are important source for yellow fillet color. In our study, Spearman rank correlation coefficients showed positive between water exchange, price and yield for both pangasius and tilapia where the correlation was larger for pangasius than for tilapia. Unlike Bangladeshi farm, high levels of water exchange are employed by Vietnamese pangasius farms produced white fillet and export to European market. The international trade are largely influenced by white vs. yellow fillet of pangasius due to its consumer preference, market implications and economic viability. Therefore, the crucial actions needed in aquaculture strategic plan for yellow to white fillet pangasius linked to international trade thus to economic development of Bangladesh.

Experimental anal infection of rainbow trout with *Flavobacterium psychrophilum*: A novel challenge model

J.K. Chettri[1,2], A. Al-Jubury[2], I. Dalsgaard[1], P.M.H. Heegaard[1] and K. Buchmann[2]

[1]National Veterinary Institute, Technical University of Denmark, Denmark
[2] Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark

Presenting author: Jiwan Kumar Chettri, jkc@sund.ku.dk

Flavobacterium psychrophilum is a Gram-negative psychrophilic bacterium causing rainbow trout fry syndrome (RTFS) in fry and are responsible for bacterial cold water disease (BCWD) in older fish. Both diseases are challenging fish welfare and economy in hatcheries and on-growing facilities. The bacteria enter hosts through gills, skin, gastro-intestinal tract and are transferred horizontally in contaminated water and vertically by sexual products. The protection afforded by injection or immersion using formalin killed whole cell bacteria has been reported but no commercial vaccine is at present available for control this pathogen. This calls for further research within RTFS vaccinology and control but when investigating vaccine efficacy and treatment a reliable challenge method is needed.

The present study was designed to evaluate and compare different infection methods including anal challenge using trout with intact skin, gills and gut. Six different challenge methods comprising intra-peritoneal injection, co-habitation, anal intubation and bath challenge (after use of stressors: hydrogen peroxide exposure and punching of tail fin or bath challenge alone) were compared and the disease development was monitored for 4 weeks. The anal intubation method produced mortality (43%) comparable to that of i.p. injected groups suggesting this new method to be preferable to i.p. injection methods as the mucosal surfaces are kept intact. Overall results from the experiment will be discussed.

Use of recombinant VHS virus encoding GFP for functional studies of anti-viral immunity in rainbow trout.

S. Shih[2], D. Sepulveda[1], V. Vakhari[3], N. Bols[4], and N. Lorenzen[1,2].

[1]National Institute of Aquatic Resources, Danish Technical University (DTU), Denmark
[2]Animal Science Institute, Aarhus University, Tjele, Denmark
[3]Institute of Marine and Environmental Technology, University of Maryland Baltimore County, Baltimore, USA.
[4]Department of Biology, University of Waterloo,Waterloo, Ontario, Canada.

Presenting author: Niels Lorenzen, nilo@aqua.dtu.dk

Viral haemorrhagic septicaemia virus or VHSV is one of the most virulent viruses occurring in farmed rainbow trout and although it is currently eradicated from the freshwater aquaculture facilities in DK, it is still causing high mortalities in rainbow trout farms in Southern European countries and in Iran. A marine reservoir of less virulent VHSV variants is furthermore present in the wild fish populations in DK and represent a constant threat against marine aquaculture.

The present study aimed at in vitro studies for improved understanding of anti-viral immunity to VHSV. In order to allow detailed time course studies of virus propagation during infection in cell culture, a recombinant variant of VHSV, encoding Green Fluorescent Protein (GFP) was established (VHSV-GFP). The recombinant virus variant propagated well in cell culture and had retained virulence to rainbow trout. The VHSV-GFP was applied in studies of the ability of the virus to cope with innate and adaptive immune mechanisms in cell culture experiments.

Time course studies in cell cultures expressing type I IFN following inoculation with the TLR3 ligand poly I:C revealed that while the cells appeared resistant to the virus shortly after poly I:C stimulation, the effect was temporary and extended incubation without poly I:C soon allowed the virus to massively infect the cells. Inoculation of susceptible cell cultures in the presence of a highly neutralizing monoclonal antibody demonstrated that the virus, possibly due to its quasispecies nature, within a single passage was able to escape from the neutralizing antibody by a point mutation in the viral glycoprotein. A robust and long lasting protective immunity thus has to be based on a timely combination of innate and polyclonal adaptive mechanisms.

Applied Hologenomics: Integrating host-microbiota interactions in fish health studies

M.T. Limborg, M. Kodama, A. Alberdi, M.T.P. Gilbert

Section for EvoGenomics, Natural History Museum of Denmark, University of Copenhagen

Presenting author: Morten Limborg, morten.limborg@gmail.com

Aquaculture is expected to provide an increasing fraction of human food, however current challenges and legislations necessitate the need for continuous development towards more sustainable production characterized by improved health and growth in farmed fish. As a contribution to this need we have recently proposed the so-called Holo-Omic framework for the study of fish health and growth by translating evolutionary thinking into an applied context. Over the past decade a new way to understand fundamental evolutionary processes has emerged that focus on the need to study a host organism and its associated microbiota together. Indeed, because all animals have evolved in intimate relationship with their associated microbiota - some of which are essential to the host metabolism - it is now becoming clear that we need to look at multicellular life forms as an interlinked holobiont unit comprised of both a host and its associated microorganisms.

Here, I will first give an introduction to our analytical framework and then cover a few disease related case examples where we envision that our Holo-Omic framework may help advance the field of aquaculture research. I will then introduce ongoing projects where we are applying the Holo-Omic framework in rainbow trout and Atlantic salmon with the aim to map key host-microbiota interactions of relevance to fish health and growth. Lastly, I will highlight limitations in these field-based projects as to bridge into a view towards future plans aimed at understanding whether (and how) the host genome controls composition and function of the gut microbiota. If indeed we are able to identify host genes associated with gut microbiota functions it may be possible to selectively breed for improved gut health in farmed fish.

Cercarial dermatitis in Danish freshwater lakes during summer 2018

A. Al-Jubury[1], E.S. Tracz[2], A. Bygum[2], P.W Kania[1] and K. Buchmann[1]

[1]Department of Veterinary and Animals Sciences, University of Copenhagen, Denmark
[2]Dermatology and Allergy Center, Odense University Hospital, Denmark

Presenting author: Azmi Al-Jubury

Temperatures in Denmark during summer 2018 were unusually high and the abiotic and biotic conditions in freshwater lakes correspondingly affected. Aquatic invertebrates and vertebrates were exposed to high temperatures which affected their physiology significantly. At the same time freshwater lakes were used to a higher extent for bathing and swimming and the increased contact between humans and the lake water had implications for human health. Several clinical cases of cercarial dermatitis were reported from different locations in Denmark and were all connected to direct contact between patients and freshwater. The disease occurs when people have direct contact with freshwater lakes containing free-swimming cercaria of bird schistosomes within the genera *Trichobilharzia* and *Bilharziella*. Birds are the definitive hosts of the parasite while freshwater pulmonate snails are the intermediate hosts.

In order to connect the clinical observations with presence of parasites in relevant locations we have performed a survey in August and September 2018. We sampled a total of 1,031 snails from different freshwater lakes during our field survey. Collected snails comprised 523 *Lymnaea stagnalis,* 440 *Radix* spp. and 65 *Planorbis* spp. from 17 Danish lakes. Snails were individually incubated in the laboratory and shedding of cercariae performed. Isolated avian schistosome cercariae were sampled for light microscopical and molecular diagnosis. Findings from the field study are discussed in the light of previous investigations and the distribution of clinical cases in 2018.

Effect of salinity on the survival and growth of pangasius catfish in southern in Southern Bangladesh

Mohammad Lokman Ali[1] and Russell Borski[2]

[1]Patuakhali Science and Technology University, Bangladesh
[2] Department of Biological Sciences, North Carolina State University, USA

Presenting author: Mohammad Lokman Ali, lokman.fri@gmail.com

The river catfish was introduced to Bangladesh in the 1990's from Thailand, and has since become a thriving aquaculture industry with over 2 million tonnes produced annually. The fish species is typically cultured in freshwater, however with the coastal waters of Southern Bangladesh being affected by salinity encroachment, we sought to determine whether catfish could be cultured in these hyposaline waters. This region is severely impacted by overfishing, and is underutilized due to increasing susceptibility to rising sea levels linked to global climate change. Thus, the aim of the present study was to assess the effect of salinity on growth and survival of *Pangasius* and in turn, determine the optimum tolerable salinity for culture of this species.

The experiment consisted of six treatments spanning a range of salinities: T_1 (0-5 ppt), T_2 (5-7 ppt), and T_4 (10-12 ppt) did not involve prior conditioning while T_3 (10-12 ppt), T_5 (12-18 ppt), and T_6 (18-22 ppt) had the fish acclimatized in 6 ppt water for 24 h prior to the start of the experiment. In T_1 (0–5 ppt) and T_2 (7–8 ppt), the survival rate was 100%. For T_3 (10-12 ppt), in which the fingerlings were first acclimatized at 6 ppt, the survival rate was also 100%. In T_4, where fingerlings were stocked directly at 10–12 ppt, survival rate was 87% with mortalities occurring as early as 5 days. At 12–15ppt (T_5), survival rate was 30% while at the highest salinity (T_6), all fingerlings died within one day. The results indicate that *Pangasius* catfish are able to survive in waters of up to 12 ppt when allowed a prior acclimation period in more dilute waters (6 ppt). A subsequent 6-month pond study evaluating growth performance of *Pangasius* catfish in different salinities (0 ppt, 6.5 ppt and 10.8 ppt) was conducted. The survival rate, weight gain, SGR, feed conversion ratio (FCR), yield, and benefit cost ratio (BCR) were similar ($P>0.05$) among the treatments. The results suggest that *Pangasius* catfish can be successfully cultured in salinities as high as 12 ppt. The ability to grow *Pangasius* in hyposaline waters of coastal Bangladesh or other regions can provide additional earnings and food sources for communities impacted by seawater encroachment linked to rising sea levels and climate change.

Associations between the skin parasite *Ichthyophthirius multifiliis* and the immune system of the fish host *Danio rerio*

LvG. Jørgensen, R. Korbut, S. Jeberg, P.W. Kania, K. Buchmann

Department of Veterinary and Animals Sciences, University of Copenhagen, Denmark

Presenting author: Louise von Gersdorff Jørgensen, lvgj@sund.ku.dk

The parasite *Ichthyophthirius multifiliis* is the causative agent of white spot disease and a major problem for the aquaculture and ornamental fish industry. It infects skin and gills of freshwater fish and cause high mortality during outbreaks. The zebrafish has become an important model to study a wide spectrum of vertebrate biological processes and many tools are available. We have therefore used adult transgenic zebrafish with Green Fluorescent Protein (GFP)-tagged neutrophils as a model to study the behaviour of these innate immune cells during a parasite assault in naïve and immunized fish. To further investigate immune mechanisms in these fish real-time qPCR was conducted. The gene expression results of naïve fish infected for the first time were characterized by an inflammatory profile whereas immunized fish showed a Th2-type reaction with elevation of antibodies and high expression of the Th2 signature cytokine IL-13. One and two days after infection both naïve and immunized fish displayed an increase of focally accumulated neutrophils in the caudal fin with the immunized fish having the highest number of cells. The number of neutrophils peaked one day after infection and decreased during the following days in naïve and protected fish. The parasite, however, continuously increased in size in fish infected for the first time and ingested more fish materials whereby the damage to the fish intensified. To investigate why the number of neutrophils decreased while the parasitosis became more severe video-recordings of the interface between the parasites and the neutrophils at the single cell level were conducted. These recordings revealed how the parasites have a way of evading and fighting the immune system of the host.

Association between stress induced by *Ichthyophthirius multifiliis* infection and differential immunological responses in skin, gills, and spleen of rainbow trout, *Oncorhynchus mykiss* (Walbaum)

K. Syahputra, P.W. Kania, A. Al-Jubury, H. Marnis, A.C. Setyawan, H. Mathiessen, K. Buchmann

Department of Veterinary and Animals Sciences, University of Copenhagen, Denmark

Presenting author: Khairul Syahputra, khairul@sund.ku.dk

Infection of rainbow trout with the parasitic ciliate *Ichthyopthirius multifiliis* induces a stress response which can be monitored by serum cortisol measurements reflecting involvement of the pituitary-interrenal cell axis in parasite infected fish. The underlying intricate physiological consequences of the stress affects have been less well elucidated-especially with regard to the differential responses in skin, gills, and spleen. The present study was conducted to elucidate the cross-talk between stress and immunological responses of rainbow trout skin, gills, and spleen following infection. A controlled experimental infection was performed and the stress reactions in the fish were elucidated by measurements of serum cortisol. Lysozyme activity and expression of immune-relevant genes skin, gills, and spleen were recorded at day 1 and 8 after parasite exposure. Infection induced a marked elevation of serum cortisol and lysozyme levels in sera and host reactions involved alteration of innate and adaptive immune defense in rainbow trout at day 8 of infection. Pro-inflammatory cytokines IL-6, IL-17 C1, anti-inflammatory cytokines IL-4/13A, IL-10, TGFβ, complement C5, chemokines CK10, CK12, acute phase proteins (precerebellin, hepcidin) and immunoglobulins (IgM, IgT) differential expression level in skin, gills, and spleen were found and correlation analyses were performed to monitor association. Results are discussed in relation to the stress-associated responses in the infected fish.

Detection of bioavailable tetracycline from pangas and tilapia aquaculture production system in Bangladesh using whole-cell biosensor bacteria

E. Ahsan[1,2], K.K. Brandt[1] and L. Ali[2]

[1] Department. of Plant and Environmental Science, Copenhagen University, Denmark

[2] Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh

Presenting author: Emranul Ahsan, meahsan@plen.ku.dk, meahsan@bsmrau.edu.bd

Antimicrobials, parasiticides, feed additives and probiotics are used in aquaculture to improve the health status of the cultured organisms and to prevent or treat disease outbreaks. However, extensive use of antibiotics results in the inhibition of susceptible organisms while selecting for the resistant ones, aquacultural use is contributing substantially to the emergence and spread of antibiotic resistance in the aquatic environment. Tetracycline is the group of antibiotics used most extensively in aquaculture for disease treatment in Bangladesh.

In this study, tetracycline (TC) concentrations were measured in pond water from 317 pangas and tilapia aquaculture systems from different regions of Bangladesh by using whole-cell biosensor bacteria (*E. coli* K12 pTetLux1). Use of WCBs is more advantageous over any other chemical analysis as it is cheap, robust and high-throughput method and suitable for rapid screening of large number of sample. To our knowledge, this is the first ever large-scale screening of tetracycline antibiotic using whole-cell bioreporters from different environmental samples in Bangladesh. A total of 79 (out of 317 samples) were found positive. The concentration of TC varied from 2.57 to 24.85µg/L. Average concentration of tetracycline detected was 1.82µg/L. The physicochemical parameters in pond water were monitored. The average values were as listed; Temperature: 30.62°C, pH: 7.88, DO: 6.32, NO_2: 2.397mg/l, PO_4: 0.24mg/l and NH_3: 0.56mg/l.

Transcriptomic profiling of Baltic cod (*Gadus morhua*) liver in response to *Contracaecum osculatum* infection

H. Marnis[1*], P.W. Kania[1], K. Syahputra[1], S. Zuo[1], H. Jensen[1], R. P. Dirks[2], K. Buchmann[1]

[1] Department of Veterinary and Animals Sciences, University of Copenhagen, Denmark
[2] Future Genomics, Leiden, The Netherlands.

Presenting author: Huria Marnis, email: huria.marnis@sund.ku.dk

Baltic cod (*Gadus morhua*) is one of the most important economic fish species and parasitic diseases of cod have implications for food safety, product quality and stock optimization which calls for a continuous monitoring of infections. During the latest decades we have observed a marked increase of infections with third-stage larvae of the anisakid nematode *Contracaecum osculatum* in Baltic cod liver. Prevalence of infection has reached 100 % in certain areas and the mean intensity has reached more than 80 parasites per host. At the same time the quality of the cod fillets have shown a decreasing trend and the mean size of cod has decreased.

In order to explain any causative connection between the infection and the physiological status of the host we have performed a transcriptomic analysis of infected cod and compared this to non-infected cod. Infected fish were collected from a local fisherman of the island Bornholm, Denmark, and a non-infected group of cod was collected from the Blue Planet, Copenhagen, Denmark. The transcriptomic analysis showed that a total of 46,577 unigenes were identified and of these 1,737 (3.7%) unigenes were differentially expressed in the infected liver of cod. Among differentially expressed unigenes (DEGs), 941 unigenes were up-regulated while 796 were down-regulated in livers infected by the third-stage larvae *C. osculatum*. The Gene Ontology (GO) enrichment analysis showed that 583 DEGs were relevant for 24, 15, and 20 Gene Ontology (GO) terms (biological process, molecular function, and cellular component, respectively). A total of 215 DEGs was involved in 685 Kyoto Encyclopedia of Gene and Genomes (KEGG) Pathways. The highest number of unigenes were involved in 1) signal transduction, 2) functions of the endocrine system and 3) 10 immune system pathways. The regulation of DEGs in those pathways and its connection to the *C. osculatum* infection of cod liver will be discussed.

Factors responsible for the off-flavour and yellow colour in *Pangasianodon hypophthalmus*

S.R. Islam[1], M.M. Haque[2] and N.O. G. Jørgensen[1],

[1]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
[2]Department of Aquaculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Presenting Author: Seikh Razibul Islam islamsr@plen.ku.dk

Over the last 20 years, production of pangasius (*Pangasianodon hypophthalmus*) in aquaculture farms in Bangladesh has significantly expanded and now contributes about 16% of the total aquaculture production of 2.3 million MT. However, pangasius cultured in Bangladesh has received less international attention than pangasius from Vietnamese fish farms. Among reasons for the limited export of Bangladeshi pangasius two important quality issues are tainting by off-flavours and yellow coloration of the flesh. The objectives of this study were to characterize off-flavour production in water of the farms and extent of off-flavours in the fish flesh, and to identify the reasons behind the yellow colouration in fish flesh. A range of studies in field ponds and experimental ponds were conducted over the last two years to assess the water quality of pangasius farmers' ponds, and to identify the reasons behind off-flavour and yellow colour in pangasius flesh by application of different water exchange frequencies and testing of various feed types in experimental ponds. The experimental work included analysis of major parameters controlling the water quality, analysis of off-flavours in pangasius flesh by GC-MS and feeds, and analysis of fish flesh by HPLC for detection of colour profiles. Two carotenoid pigments (lutein and zeaxanthin) that impart yellow or orange colour to many common foods were found in yellowish pangasius flesh. The pigments originated from different feed ingredients being used to formulate pangasius feeds. Maize is the most commonly used (5-20%) fish feed ingredient in Bangladesh, and feeds were determined to contain lutein and zeaxanthin at an amount of about 640 µg per 100 g feed. Off-flavours profiles of the fish are still being studied, but preliminary studies suggest that certain phytoplankton organisms, especially cyanobacteria, may be main responsible for the tainting, but more analyses are needed before a general conclusion can be given. A clear relation between ingredients of pangasius feed and the muscle coloration was observed. An on-going pond experiment shows that replacement of intense coloured feed ingredients to low-pigment containing feeds most likely will result in white flesh colour. Conclusions on the extent of off-flavouring of the fish await analysis at University of Copenhagen.

Detection of bioavailable Arsenic from pangas and tilapia farms in Bangladesh fed with groundwater by using bioluminescent biosensor bacteria (*E. coli* K12 pJAMA arsR)

Muhammad Abdur Razzak[1], Md. Emranul Ahsan[2], Md. Mohaimen Khan Yesa[1] and Kristian Koefoed Brandt[2],

[1]Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh
[2]Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Presenting Author: Muhammad Abdur Razzak, arazzak@pstu.ac.bd

Long term exposure to arsenic through drinking water as well as food raised in As contaminated water poses serious health hazards. The safety limit for arsenic in drinking water for most European countries and United States is 10 µg/l; elsewhere like in Bangladesh is 50 µg/l. So, it is essential to determine the As concentration of water body in severely As contaminated region to justify whether the products raise in those regions are safe or not with considering the water quality.

In this study, water samples were collected from selected ponds located at Barura, Bakra, Chandina, Jhikorgacha, Kachua, Kotalipara, Sachar, Trishal and Tala during the period of January 2017 to May 2018. Most of the ponds are used for commercial fish culture whether the average age of the ponds was approximately 10 years and the average depth of the ponds was got about 1.72 meter. Rain water is the main source for cultivation but farmers also use shallow tube well's water during drought.

Highest level of AsV and AsIII were found at Kotalipara which were 1887.18 µg/l and 14.19 µg/l and lowest figure were 146. 72µg/l and 2.91µg/l respectively found at Rajoir under Madaripur. Within the total sample only 6.13% is above 5 µg/l which is still in tolerance level both for As affected water body and As concentrations in water body because no catastrophe was enlisted by any researcher in this concentration of As, more specifically, fishes. Calculated average value of temperature, DO, pH, ammonia, nitrate and phosphate of all water samples were 30.7±0.77°C, 6.24±1.30 mg/l, 7.5±1.37, 0.29 mg/l, 2.45 mg/l and 0.22 mg/l, respectively, which indicates the safe aquatic environment of the experimental ponds. So, it can be stated that, pangas and tilapia raise in the experimental ponds are safe for consumption.

Ecological and molecular links between *Contracaecum osculatum* (Nematoda, Anisakidae) in Baltic cod (*Gadus morhua*) and grey seal (*Halichoerus grypus*)

S. Zuo, P.W. Kania, A. Al-Jubury, N. Daugbjerg Christensen, F. Mehrdana, Q. Bahlool, R. Korbut, B. Huwer, M.H. Marana, K. Buchmann

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Presenting author: Shaozhi Zuo, chao@sund.ku.dk

Baltic cod (*Gadus morhua*) is one of the most important commercial fish in the Baltic Sea. However, in the last decades it has been shown that the infection level of the nematode *Contracaecum osculatum* (3rd stage larvae) has experienced a marked increase in Baltic cod. Previous studies have shown that the increasing infection level is related to the increasing population of grey seal (*Halichoerus grypus*) in the Baltic area. These seals harbor heavy loads of adult stages of the nematode in their stomach. This has now been demonstrated by classical helminthological methods and confirmed by molecular methods. The study has reflected how cod become infected in the Baltic sea. We investigated if this specific parasite could be found in grey seals, cod, sprat and linked this to the life cycle involving copepods as the first transport host, small pelagic fish such as sprat (feeding on copepods) as the secondary transport host and cod as the third transport host before seals ingest cod and obtain infection. A total of 100 small benthic organisms including *Harmothoë sarsi* and *Saduria entomon* were studied as well but we did not find trace of the parasite in these benthic invertebrates. Morphological identification and molecular study of the *C. osculatum* (internal transcribed spacer (ITS) and mitochondrial genes) indicated that smaller cod obtain infection when they start feeding on small pelagic fish such as sprat. Prevalence of infection in sprat was 11.6% with an intensity of 1-8 third stage larvae of *C. osculatum* per fish.

Effect of hydrogen peroxide, polycyclic musks and salinity on aquatic organisms

Md. Rushna Alam[1], Friday Ojie Ehiguese[2], M. Laura Martin-Diaz[2]

1Department of Aquaculture, Faculty of Fisheries, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh.
2Physical Chemical Department, University Institute of Marine Research (INMAR), International Campus of Excellence of the Sea (CEI.MAR), University of Cadiz, República Saharaui s/n, 11510 Puerto Real (Cádiz), Spain.
Presenting author: Rushna Alam, rushna_alam@pstu.ac.bd

Environmental concentration of pollutants due to natural and anthropogenic causes are of paramount importance as far as marine and coastal organisms are concerned. Pollutants such hydrogen peroxide, polycyclic musks and even salinity may impair the normal life of aquatic organisms which can be revealed by analyzing a battery of biomarkers. One of our study aimed to determine the effects of hydrogen peroxide (0.05, 0.5, 5, 50 and 500 µM) on larval development of the Mediterranean mussel *M. galloprovincialis* and to examine a set of cellular parameters (biomarker) on the clam *R. philippinarum* after 21-days of exposure. In our short-term toxicity study we demonstrated that increased concentration of hydrogen peroxide, including environmentally relevant levels, might have toxic effects on mussels during their early life stages ($p < 0.05$). Hydrogen peroxide induced oxidative stress in clams which was indicated by the activation of antioxidant enzymes, significant increase in LPO levels ($p < 0.05$) and significant decrease in TL ($p < 0.05$) in the digestive gland tissues. *R. philippinarium* exposed to 0.005, 0.05, 0.5, 5 and 50 µg/L of polycyclic musks (galaxolide (HHCB) and tonalide (AHTN)) for 21 days and HHCB and AHTN significantly induced EROD and GST enzymatic activities ($p<0.05$) at environmental concentrations. These substances induced GPx activity and significantly inhibited GR activity ($p<0.05$). All concentrations induced significant increase of LPO on day 21 for both substances leading to DNA damage. Although these substances have been reported none acutely toxic, this study have shown they induce oxidative stress and consequently, genetic strands break in marine organisms. In another attempt, embryo-larval bioassay and mortality test of fry and juvenile were performed to know the effect of salinity (0, 2.5, 5.0 and 7.5 ppt) on the freshwater fish species *O. pabda*. The percentage of normal larvae were significantly lower ($p<0.05$) at salinity 2.5, 5.0 and 7.5 ppt whether significantly higher fry mortalities ($p<0.05$) were observed at 5.0 and 7.5 ppt salinity.

List of participants

Surname	First name	e-mail	Affiliation
Ahsan	Emranul	meahsan@bsmrau.edu.bd	Patuakhali Science and Technology University, Bangladesh
Alam	Rushna	rushna_alam@pstu.ac.bd	Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh
Ali	Mohammad Lokman	lokman.fri@gmail.com	Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh
Al-Jubury	Azmi	azmi@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Andreassen	Jørn	jandreassen@webspeed.dk	Faculty of Science, University of Copenhagen, Denmark
Buchmann	Kurt	kub@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Børsting	Ejner	ejner@borsting.dk	Bjæverskov, Denmark
Chettri	Jiwan Kumar	jkc@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Dalsgaard	Inger	inda@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Donati	Valentina	valdo@aqua.dtu.dk	National Veterinary Institute, Technical University of Denmark
Hansen	Mie J.	mijhan@aqua.dtu.dk	National Institute of Aquatic Resources, Technical University of Denmark
Hoque	Sazedul	sazedul.fst@pstu.ac.bd	Department of Fisheries Technology, Patuakhali Science and Technology University, Bangladesh
Islam	Seikh Razibul	islamsr@plen.ku.dk	Section of Microbial Ecology and Biotechnology, University of Copenhagen, Denmark
Jørgensen	Louise von Gersdorff	lvgj@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Jørgensen	Niels O.G.	nogj@plen.ku.dk	Microbial Ecology and Biotechnology, University of Denmark
Kania	Per	pwk@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Kodama	Miyako	miyako.kodama@bio.ku.dk	Department of Biology, University of Copenhagen

Surname	First name	e-mail	Affiliation
Limborg	Morten Tønsberg	morten.limborg@gmail.com	Natural History Museum of Denmark, University of Copenhagen, Denmark
Lorenzen	Niels	nilo@vet.dtu.dk	Department of Animal Science - Fish Health, Aarhus University, Denmark
Madsen	Lone	loma@vet.dtu.dk	National Veterinary Institute, Technical University of Denmark
Mahmud	Sultan	sultanmahmud77@yahoo.com	Bangfish Project, Bangladesh
Marana	Moonika Haahr	mmarana@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Marnis	Huria	huria.marnis@sund.ku.dk	Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Mathiessen	Heidi	heidimat92@gmail.com	Aquatic Pathobiology, University of Copenhagen, Denmark
Mazaheri	Sanaz	shma@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Mehrdana	Foojan	foojan@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Patel	Sonal	sonal.patel@imr.no	Institute of Marine Research, Bergen, Norway
Plambech	Marie	mpla@aqua.dtu.dk	National Institute of Aquatic Resource, Technical University of Denmark
Razzak	Muhammad Abdur	arazzak@pstu.ac.bd	Department of Aquaculture, Patuakhali Science and Technology University, Bangladesh
Schmidt	Jacob Günther	jacsc@aqua.dtu.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Setyawan	Agung Cahyo	agung@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Skov	Jakob	jask@sund.ku.dk	Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Sokolova	Maria	apple8150@yandex.ru	National Institute of Aquatic Resources, Technical University of Denmark, Denmark
Syahputra	Khairul	khairul@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Sørensen	Jonas Steenholdt	xzd234@alumni.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Tange	Esther	wqn604@alumni.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark
Zuo	Shaozhi	chao@sund.ku.dk	Aquatic Pathobiology, University of Copenhagen, Denmark