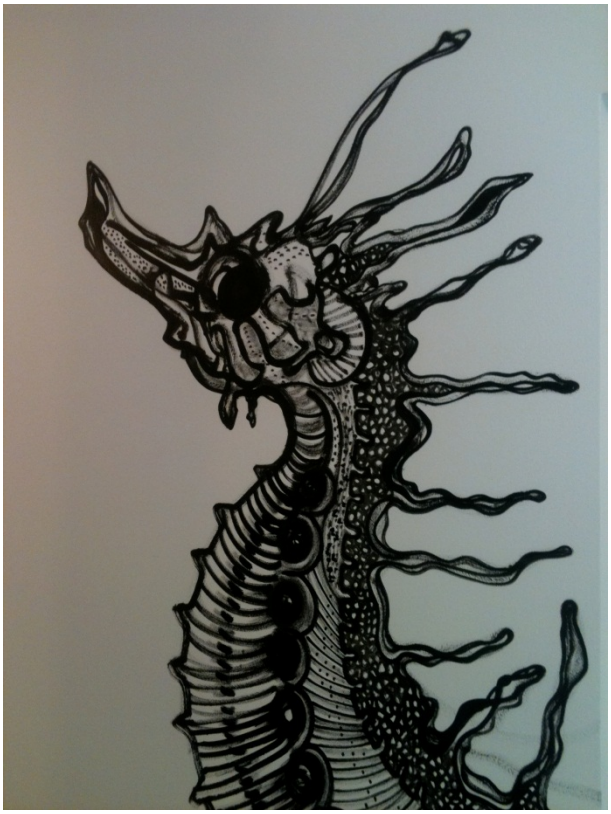
DAFINET WORKSHOP

AND

PH.D. COURSE (4 ECTS)



Illustrations by Maria Dubin

DIAGNOSIS AND CONTROL OF FISH DISEASES

April 9th to 11th, 2013

Venue:

Lecture Theatre 1-01
Bülowsvej 17
1870 Frederiksberg
Denmark

In association with:

AquaTNET
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University of Copenhagen
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Book of abstracts

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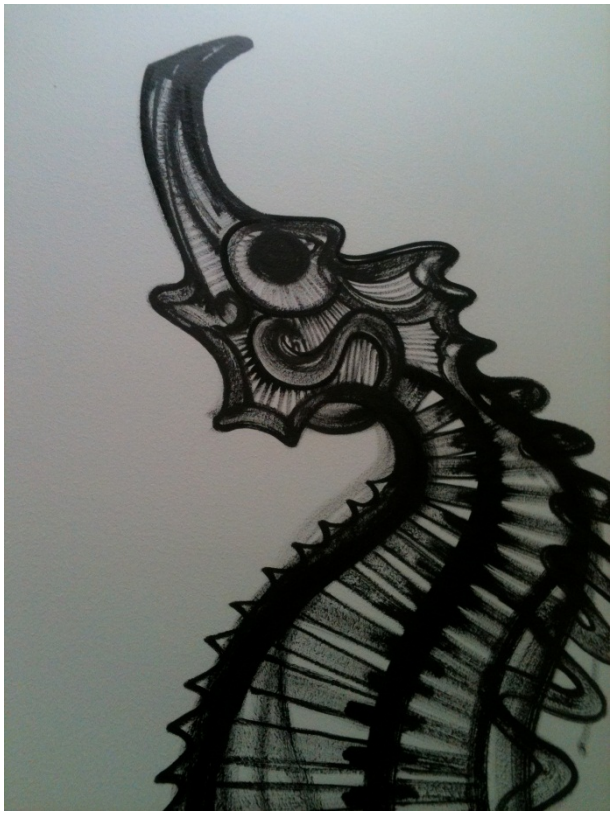

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Program
Tuesday April 9th, 2013

10.00	Welcome address by DAFINET centre and research school leader Kurt Buchmann *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark*
10:15	Professor Barbara Nowak *National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Australia* Fish diseases down under
11.00	Professor Kurt Buchmann *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Emerging *Pseudoterranova decipiens* problems in Baltic cod (*Gadus morhua* L.) associated with increasing seal populations
11.15	Postdoc Jakob Skov *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Monitoring parasite infections in maricultured rainbow trout
11.30	Ph.D. student Helene Strøm *Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences,University of Copenhagen, Denmark* Effect of commercial and experimental vaccines against enteric redmouth disease (ERM) in rainbow trout challenged by waterborne Y*ersinia ruckeri* o1 biotype 2 infection
11.45	Research Scientist Katy Urquhart *Scottish Fish Immunology Research Centre, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB242TZ, Scotland, UK* Development of a non-lethal sampling method to monitor immune response and disease progression following infection with infectious salmon anaemia virus in Atlantic Salmon (*Salmo salar* L.)
12.00	**Lunch break**
13.00	Postdoc Jiwan Kumar Chettri *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* PAMP induced expression of immune relevant genes in head kidney leukocytes of rainbow trout (*Oncorhynchus mykiss*)
13:30	Ph.D. student Sidhartha Desmukh *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* *Yersinia ruckeri* infection of rainbow trout: Entrance portals and spread in the host
14:00	**Coffee break**

14:30 Professor Ken MacKenzie
University of Aberdeen, Scotland
Parasites and fisheries

15.00 Postdoc Louise von Gersdorff Jørgensen
Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark
Immunity in fish against white spot disease: Prospects for vaccines

15.30 Ph.D. student Bartolomeo Gorgoglione
Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Aberdeen, UK
Transcriptomic assessment of the immune response modulation by PKD/VHS co-infections in brown trout

15.45 Ph.D. student Kasper Rømer Villumsen
Section of Veterinary Clinical Microbiology, Institute of Veterinary Disease Biology, University of Copenhagen
Understanding vaccine-induced protective mechanisms against infection with *Aeromonas salmonicida* subsp. salmonicida in rainbow trout

16.00 Ph.D. student Sune Riis Sørensen
National Institute of Aquatic Resources,
Technical University of Denmark, Denmark
Microbial interference with hatch and survival of European eel larvae

16.15 Ph.D. student Dennis Bela-ong
Section for Fish Diseases, National Veterinary Institute,
Technical University of Denmark, Denmark
Do micro RNAs induced by *VHSV* virus in rainbow trout (*Oncorhynchus mykiss*) possess anti-viral activity?

16.30 Ph.D. student Per Jepsen
Department of Environmental, Social and Spatial Change,
Roskilde University, Universitetsvej 1, DK-4000, Roskilde, Denmark
Identification and expression of heat-shock protein 70 and ferritin in embryos of the calanoid copepod *Acartia tonsa* during transition between subitaneous and quiescent state

16.45 Discussion and conclusion

Program
Wednesday April 10th, 2013

10.00	Professor Barbara Nowak *National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Australia* Amoebic gill disease
10.30	Ph.D. student Qusay M. Bahlool *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Immune regulating ES products in parasitic nematodes
11.00	Postdoc Celia Agusti *Section for Parasitology and Section for Immunology, Norwegian Veterinary Institute, Oslo, Norway* Searching for vaccine candidates against salmon louse (*lepeophtheirus salmonis*)
11.30	Research assistant Diana Sindberg *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Occurrence of salmon lice in wild and cultured fish populations
11.45	Postdoc Ole Sten Møller *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Knowing the weapons of your opponents
12.00	**Lunch break**
13.00	Associate Professor Per Kania *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Serum Amyloid A gene expression and immunohistochemical localization in rainbow trout, *Oncorhynchus mykiss*, infected by *Yersinia ruckeri*
13.30	Postdoc Jakob Skov *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Immune stimulating compounds and their use in fish production
13.45	Ph.D. student Simon Haarder *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Zebra fish as a model for inflammatory bowel disease (IBD)
14:00	**Coffee break**

15.00	Research leader Uwe Fischer *Friedrich-Loeffler Institute Insel Riems, Germany* Cellular immune reactions in rainbow trout
15.45	M.Sc. Veronica Soto-Lampe *Friedrich-Loeffler Institute Insel Riems, Germany* Characterization of peptides loaded into MHC class I in IHNV infection of rainbow trout (*Oncorhynchus mykiss*)
16.00	Ejner Børsting *Bjæverskov, Denmark* Selective breeding for liveability in a commercial breeding programme
16.30	Postdoc Hans-Christian Ingerslev *National Veterinary Institute,* *Technical University of Denmark, Frederiksberg C, Denmark* Is the intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*) influenced by diet type and challenge by Y*ersinia ruckeri*?
16.45	Ph.D. student Karina Rasmussen *Department for Cancer and Inflammation Research,* *University of Southern Denmark, Odense, Denmark* Comparative analysis of immune cells of the primary and secondary vascular system in rainbow trout *Oncorhynchus mykiss* (Walbaum)
17.00	Ph.D. student Rói Christiansen *National Veterinary Institute,* *Technical University of Denmark, Frederiksberg, Denmark* Detection and quantification of *Flavobacterium psychrophilum* specific bacteriophages in rainbow trout upon different administration methods: Implications for disease control in aquaculture
17.15	Discussion and conclusion
18.00	**DAFINET buffet at Stigbøjlen 7, 1870 Frederiksberg**

Program
Thursday April 11th, 2013

10.00	Professor Barbara Nowak *National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Australia* Vaccination strategies in salmonid aquaculture
10.45	Professor Ken MacKenzie *University of Aberdeen, Scotland* Parasites as biological tags
11.15	Research assistant Foojan Mehrdana *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Infection of North Sea cod (*Gadus morhua* L.) postlarvae and juveniles with the parasites *Hysterothylacium aduncum* Rudolphi and *Caligus* sp.
11.30	Ph.D student Rasmus D. Heinecke *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark* Immune response of rainbow trout larvae against *Ichthyophthirius multifiliis*
12.00	**Lunch break**
13.00	Section leader Niels Lorenzen *Department of Poultry, Fish and Fur Animals; National Veterinary Institute, Technical of University Denmark* Antiviral immunity in fish – Functional analysis using DNA vaccination as a tool
13.30	Postdoc Mikkel-Ole Skjoedt *Department of Clinical Immunology, University Hospital of Copenhagen, Denmark* Biology of Map-1, a novel regulatory member of the complement system that attenuates myocardial ischemic reperfusion injuries and inhibits arterial thrombogenesis *in vivo*
13.45	Postdoc Maki Otani *Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark* Three-dimensional imaging analysis of *Yersinia ruckeri* infected rainbow trout (*Oncorhynchus mykiss*) gills by optical projection Tomography
14.00	Artist and painter Maria Dubin, Copenhagen Denmark Seahorse Symphony – Why fish never cease to inspire
15.00	**Final discussion and closing the DAFINET meeting**

Abstracts

Fish diseases down under

Nowak B. F.

National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Launceston, Tasmania, Australia

Diversity of Australian mariculture reflects a wide range of environmental conditions and high biodiversity of the marine environment. The main fish species farmed include Atlantic salmon, rainbow trout, Southern Bluefin Tuna, yellowtail kingfish and barramundi. As many of these species are farmed in grow-out cages, they can be easily affected by parasitic diseases. Epizootics of a range of different taxa of metazoan parasites have been documented in recent years among southern bluefin tuna ranched in southern Spencer Gulf, South Australia. These parasites include blood flukes (*Cardicola* spp.) and sea lice (primarily *Caligus chiastos*). Sea lice are one of the most significant health problems in mariculture worldwide. However, sea lice do not cause any significant issues in Australian mariculture. In contrast, *Cardicola* spp. is considered a significant pathogen in southern bluefin tuna. Bacterial diseases can also affect farmed fish, for example yersiniosis can be a problem in salmon hatcheries. Epitheliocystis is a bacterial infection which has been reported from over 80 species of fish both from marine and freshwater environment and can cause mortalities in aquaculture. Understanding fish diseases will results in development of effective control methods

Presenting author: Barbara Nowak; bnowak@amc.edu.au

Emerging *Pseudoterranova decipiens* problems in Baltic cod (*Gadus morhua* L.) associated with increasing seal populations

Buchmann K. and Kania P.W.

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark

Cod worms (seal worms) *Pseudoterranova decipiens* are prevalent anisakid nematode larvae in many cod stocks in the Atlantic and the Pacific but have until recently been absent or extremely rare in the isolated and stationary Baltic cod population located in the Baltic Sea between Denmark Germany, Poland, Sweden, Finland, Russia and the Baltic republics. This may be associated with a previously low population of the final hosts (seals) in the main spawning grounds of this Baltic fish stock. During the latest decade, however, the Baltic has been invaded successfully by grey seals (*Halichoerus grypus*) and in 2012 more than 250 grey seals were colonizing the small rocky island group Ertholmene located east of Bornholm in the Southern Baltic. Local fishermen complained subsequently about seals excessively harvesting fish directly from fishing gear. Cod fillets originating from fish caught in the area were investigated for the presence of nematodes and it was found that some of the fillets were infected by third stage larvae of *Pseudoterranova decipiens*. The intensity (2-4 worms per fish) is still low compared to other marine areas but the risk for further propagation and spreading of worms should be noted and possibly prevented.

*Presenting author:*Kurt Buchmann; kub@sund.ku.dk

Monitoring parasite infections in maricultured rainbow trout

Skov J., Sindberg D., Mehrdana F., Jaafar R. M., Marana M. H., Jensen H. M., Kania P. W. and Buchmann K.

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark

The food hygienic standard of rainbow trout (*Oncorhynchus mykiis*) from Danish mariculture was evaluated regarding the status of infection with zoonotic nematode larvae belonging to the family Anisakidae. Thus, during slaughter in November and December 2013, a total of 100 rainbow trout (10 fish from each of 10 Danish mariculture facilities) were examined for the presence of anisakid larval stages in the body cavity (all peritoneal surfaces) by macroscopical inspection and in the belly flap musculature by pepsin digestion. Half of the sampled rainbow trout (n = 50) had a total body weight ranging from 0.383 to 1.168 kg and were characterized by pale or poorly colored flesh and no or limited fat deposits in the abdominal cavity indicating a low intake of pelleted feed. The body weight of the remaining half of the sample (n = 50) ranged from 1.216 to 3.782 kg and these fish showed colored flesh and medium to heavy fat deposits reflecting a high food intake dominated by pelleted feed. Assessment of the stomach content revealed that 26% of the smaller rainbow trout had eaten fish, crustaceans, and molluscs. Despite this finding, all rainbow trout were negative for nematode larvae in the peritoneum and belly flap musculature indicating no transmission of zoonotic anisakids and a high food hygienic standard in this regard.

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

Effect of commercial and experimental vaccines against enteric redmouth disease (ERM) in rainbow trout challenged by waterborne *Yersinia ruckeri* O1 biotype 2 infection

Strøm H.K., Aalbæk B., Otani M., Villumsen K.R., Neumann L. and Raida M.K.

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

In recent years, there has been an increase in reported outbreaks of enteric redmouth disease (ERM) in ERM vaccinated farmed rainbow trout, which have been associated with non-motile, virulent serovar O1 strains of *Yersinia ruckeri* classified as biotype 2. A standardised challenge model has been developed based on a newly isolated and highly virulent *Y. ruckeri* O1 biotype 2 strain obtained from an ERM disease outbreak in a Danish trout farm. This waterborne infection model gives us the opportunity to test and evaluate the effect of commercial and experimental vaccines against *Y. ruckeri* O1 biotype 2. An experimental immersion, bath and intraperitoneal (i.p.) injection vaccine containing equal amounts of *Y. ruckeri* O1 biotype 1 and biotype 2 were developed, based on the most immunogenic strains collected from various ERM disease outbreaks. The effect of the experimental vaccine has been compared to a state-of-the-art commercial ERM immersion vaccine (AquaVac® Relera™). Un-vaccinated and sham vaccinated rainbow trout were included as controls. Two months post vaccination the groups were challenged in duplicate with *Y. ruckeri* O1 biotype 2 by bath. No effect of the experimental immersion or bath vaccine was observed in the present study. However, full protection was achieved with i.p. injection of the experimental vaccine. Bath vaccination with AquaVac® Relera™ induced a significant, partial protection relative to the control groups. It is suggested that the immunity induced by immersion and bath vaccination is not sufficient to protect the rainbow trout fry since mortalities are still high in the vaccinated groups, which may explain the incidences of ERM disease outbreaks due to high virulent biotype 2 strains in fish farms where all the fish are immersion vaccinated.

Presenting author: Helene K. Strøm; helst@sund.ku.dk

Development of a non-lethal sampling method to monitor immune response and disease progression following infection with infectious salmon anaemia virus in Atlantic salmon (*Salmo salar* L.)

Urquhart K.[1], Monte M.[2], Secombes C.J.[2]and Collet B.[1]

[1]*Marine Scotland Science, Marine Laboratory, 375 Victoria Road, Aberdeen, AB119DB, Scotland, UK*

[2]*Scottish Fish Immunology Research Centre, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB242TZ, Scotland, UK*

Traditional fish challenge experiments are carried out using large numbers of fish which are killed at regular intervals during an infection, with parameters such as pathogen load and/or immune parameters being measured. Since the stage of the disease is unknown when the fish is sampled, the pathogen load is extremely variable between individuals resulting in a poor description of disease progression and host immune repsonse.

We have developed a non-lethal infection model in order to reduce the number of fish required and to improve greatly the scientific information generated. An infection challenge model using infectious salmon anaemia virus (ISAV) was carried out and the pathogen load and immune parameters measured using repeat bleeding of the same individual at a number of time points throughout the infection cycle.

The results reveal that it is possible to follow an individual's response throughout the infectious challenge using qPCR, and flow cytometry analysis. The relationship between immune and welfare parameters and pathological status may help predict the risk of pathogen infections in fish farms. In addition to contributing to the 3Rs principle, this project has the potential to improve the quality of scientific output in fish disease research.

Presenting author: Katy Urquhart; katy.urquhart@scotland.gsi.gov.uk

PAMP induced expression of immune relevant genes in head kidney leukocytes of rainbow trout (O*ncorhynchus mykiss*)

Chettri J.K., Raida M.K., Holten-Andersen L., Kania P.W. and Buchmann K.

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark

Host immune responses elicited by invading pathogens depend on recognition of the pathogen by specific receptors present on phagocytic cells. However, the reactions to viral, bacterial, parasitic and fungal pathogens vary according to the pathogen-associated molecular patterns (PAMPs) on the surface of the invader. Phagocytic cells are known to initiate a respiratory burst following an exposure to the pathogen, but the underlying and associated specific elements are poorly elucidated in fish. The present study describes the differential response of head kidney leukocytes from rainbow trout (*Oncorhynchus mykiss*) to different pathogen associated molecular patterns mimicking viral (poly I:C), bacterial (flagellin and LPS) and fungal infections (zymosan and β-glucan). Transcript of cytokines related to inflammation (IL-1β, IL-6, IL-10 and TNF-α) were highly up-regulated following LPS exposure whereas flagellin or poly I:C induced merely moderate reactions. In contrast, IFN-γ expression was significantly higher in the poly I:C stimulated group compared to LPS group. When head kidney cells were exposed to zymosan or β-glucan, genes encoding IL-1β, TNF-α, IL-6 and IL-10 became up-regulated. Their level of up-regulation was comparable to LPS but the kinetics differed. In particular, TNF-α induction was considerably slower when stimulated with zymosan or β-glucan. The gene encoding COX-2 enzyme, which is a central element in initiation of inflammatory reactions, was significantly higher in stimulated cells. But a depressing effect of high concentrations of LPS and zymosan became evident after 4 h exposure. This study suggests that rainbow trout leukocytes respond differently to viral, bacterial and fungal PAMPs, which may reflect activation of specific signaling cascades eventually leading to activation of different immune effector molecules.

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

Yersinia ruckeri infection of rainbow trout: entrance portals and spread in the host

Deshmukh S.[1] Khimmakthong U.[2], Chettri J.K.[1], Bojesen A.M.[3], Kania P.W.[1], Dalsgaard I.[4] and Buchmann K.[1]

[1] *Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Stigbøjlen 7, Frederiksberg C 1870, Denmark.*

[2]*Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.*

[3] *Section of Microbiology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Stigbøjlen 4, Frederiksberg C 1870, Denmark.*

[4] *National Veterinary Institute, Technical University of Denmark Bulowsvej 27, 1870 Frederiksberg C, Denmark*

The portal of entrance of either formalin inactivated or live *Yersinia ruckeri* organism in rainbow trout fish was studied by applying immunohistochemistry and *in-situ* hybridization. The sequential study involved a specific monoclonal antibody and a specific oligonucleotide probe binding to *Yersinia ruckeri.* It demonstrated the differential and regional uptake of both formalin inactivated and live bacterial organism in rainbow trout. The uptake dynamics in various organs/tissues demonstrated a site specific propensity between formalin inactivated and live bacteria. The possibility that lateral lines, dorsal fins and the gastro-intestinal tract could act as an active avenue to bacterial entrance was shown by both immunohistochemistry and *in situ* hybridization. The translocations of systemically absorbed formalin inactivated and live bacteria within different host body compartments were elucidated.

Presenting author: Sidhartha Deshmukh; sid@sund.ku.dk

Parasites and fisheries

MacKenzie K.

School of Biological Sciences (Zoology), University of Aberdeen, Aberdeen, Scotland, U.K.

Two different aspects of the relationship between parasites and marine fisheries are described and discussed: the effects of parasites on fisheries, and the effects of fisheries on parasites. Parasites can have negative effects on fisheries through loss of biomass due to pathogenic parasites, or through highly visible "spoilage" parasites reducing the market value of the product. While the effects of parasites on fisheries has been fairly well documented, little consideration has been given to the effects of overexploitation by marine fisheries on parasite biodiversity. Two examples from the northeast Atlantic are given of significant decreases in levels of infection of two different helminth parasites with increasing fishing intensity, one possibly resulting in total extinction and the other in local extinction of the parasite.

Presenting author: Ken MacKenzie**;** k.mackenzie@abdn.ac.uk

Immunity in fish against white spot disease: prospects for vaccines

Jørgensen Lv.G.

Laboratory of Aquatic Pathobiology, Section of Parasitology and Aquatic Diseases, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark

The protozoan parasite *Ichthyophthirius multifiliis* (Ich), causing white spot disease, infects most species of freshwater fish and poses a major problem for the aquaculture and ornamental fish industry. Control of the disease may be obtained by repetitive treatments using chemicals. However, a prophylactic approach would be an economically and environmentally superior alternative. Fish - surviving an infection - are able to acquire a high level of immunity against Ich and during the last 40 years the mechanisms responsible for this immunity have been studied. For a long time, focus has been placed on humoral immune elements due to the fact that immune fish respond with a high IgM titer specific for Ich. These antibodies are furthermore capable of immobilizing the parasite. The parasite protein (I-antigen) to which the antibodies bind has also received a great deal of attention. However, recent research has shown that in the gills of immune fish mucosal immunoglobulin IgT may play an even larger role than IgM. This recently discovered antibody seems to primarily function at mucosal surfaces and because the parasite only invades fish skin and gills it is tempting to speculate that IgT might be the most important immune factor protecting the fish against Ich. Differences in IgM and IgT responses in skin and gills, however, do exist.

A high protection level against Ich has been obtained in channel catfish (*Ictalurus punctatus*) with a vaccine consisting of a purified I-antigen injected together with Freund's complete adjuvant. We are in a new project (NAPVAC) investigating the possibility of making a vaccine consisting of recombinant bacteria expressing Ich proteins. Details of the preliminary investigations will be presented.

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

Transcriptomic assessment of the immune response modulation by PKD/VHS co-infections in brown trout

Gorgoglione B.[1;2], Taylor N[2]., Martin S.A.M:[1],Feist S.[2] and Secombes C.J.[1]

[1]*Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Aberdeen, UK*

[2]*CEFAS, Weymouth Laboratory, Weymouth, Dorset, UK*

It is common for a variety of micro- and macro-parasitic species to simultaneously infect animals in the wild, as well as in farm environments. Animal disease studies are focused on examining the impact of single infections, and, despite a growing awareness, co-infection studies are still very limited, mainly to humans where there are some well established models. Proliferative Kidney Disease (PKD), caused by a myxozoan parasite, *Tetracapsuloides bryosalmonae,* is an emergent economically important disease of salmonids in Europe and North America. Viral Haemorrhagic Septicemia (VHS), caused by a *Novirhabdovirus*, is an OIE notifiable listed disease; world-wide regarded as one of the most economically important for wild and cultured fish, resulting in high morbidity and mortality. Brown trout (*Salmo trutta*), the European native trout species, are susceptible to both PKD and VHS but no information is available as to how their immune system reacts in response to multiple infection. This study aims to gain a better understanding as to how the brown trout immune system is modulated during a co-infection when compared to single infections. Naturally PKD-infected brown trout, after a seasonal outbreak on a fish farm, were experimentally bath challenged with VHSV-Ia in a biosecurity facility. Typical histopathological lesions were observed for each disease. Specifically designed RT-qPCR surveys were carried out on kidney samples to assess firstly the individual pathogen burden and to detect the host immune response in terms of key marker gene expression. Pathogen screening allowed groups selection, with distinction between VHS+/PKD- and VHS+/PKD+ (co-infected) fish. The modulation of pro-inflammatory and antimicrobial peptide genes was confirmed to be strongly driven by the viral infection related to the parasite infection. Early activation of cellular and humoral responses was detected in co-infected fish with, in general, a stronger up-regulation of Th-1 and antiviral markers. Interestingly, fish resistant to both infections showed a rapid and significant induction of Th-1, Th-2 and antiviral response marker genes. Furthermore, transcriptomic analysis was carried out using an Atlantic salmon designed oligonucleotide microarray (Salar_2, Agilent 4x44K platform) to better assess the differential immune gene expression between single- and co-infected fish.

Presenting author: Bartolomeo Gorgoglione: b.gorgoglione@abdn.ac.uk

Understanding vaccine-induced protective mechanisms against infection with A*eromonas salmonicida* subsp. *salmonicida* in rainbow trout

Villumsen K.R. and Raida M.K

Section of Veterinary Clinical Microbiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark

Efficient prophylactic measures against *Aeromonas salmonicida* subsp. *salmonicida* has been a focus of international research for more than 70 years. Oral, immersion and bath immunizations have been attempted, before the focus finally settled on the intraperitoneal injection of mineral oil-adjuvanted bacterins in the early 1990's. In a previous study on the protective effect of such injectable vaccines, performed in collaboration with co-workers, we have showed that both a commercial and an experimental vaccine induced significantly reduced mortality during an experimental bath-challenge. Furthermore, we found a significant, positive correlation between the levels of circulating, specific antibodies and endpoint survival, as well as a significant depletion of antibodies during the early phase of infection in vaccinated fish, indicating a strong humoral immune response as a successful protective mechanism against infection with *A. salmonicida*. However, we now present results from bath vaccination trials, demonstrating significantly reduced levels of mortality (P=0.005 and P=0.019, for single and dual immunization, respectively), when compared to un-vaccinated controls after an experimental bath-challenge. Despite relatively high levels of protection (RPS≈80%), none of the vaccinated groups display significant induction of circulating specific antibodies, arguing against a humoral immune response as the main protective mechanism in the bath vaccinated fish. These results indicate that the nature of the protective mechanism induced by vaccination against *A. salmonicida* depends on the route of administration, and that effective, long-lasting protection can be achieved through different mechanisms.

Presenting author: Kasper Rømer Villumsen; krv@sund.ku.dk

Microbial interference with hatch and survival of European eel larvae

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Recent research has significantly improved our knowledge and capabilities in the field of *in vitro* production of yolk sac larvae from European eel (*Anguilla anguilla*). Female broodstock European eels are matured by weekly administration of pituitary extract and male eels with hCG (human chorionic gonadotropin), which afford gametes for *in vitro* fertilization studies. The maturing process may lead to mass hatchings of up to ½ million larvae of which some survive the entire yolk sac phase. However, the rearing of larvae suffers from high larval mortalities, and water quality might be a crucial factor for larval survival in rearing systems.

By applying antibiotic treatment as a research tool, it was possible to determine the extent of microbial interference in the production of high numbers of good quality larvae. By controlling microbiota during egg and larval incubation, the egg hatching success and larval longevity more than doubled. Using scanning electron microscopic analysis it was observed that microbe inhibiting treatments reduced bacterial colonization of the eggs surface, which possibly cause reduced gas and ionic exchange across chorionic membrane.

These results suggest that future eel larviculture should not only focus on optimizing physical incubation conditions, but certainly also on the control over microbial interference.

Presenting author: Sune Riis Sørensen; srs@aqua.dtu.dk

Do microRNAs induced by V*iral Hemorrhagic Septicemia* virus in rainbow trout (*Oncorhynchus mykiss*) possess anti-viral activity?

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

Microribonucleic acids (miRNAs) are small (18-22 nucleotides) endogenous RNAs that potently regulate the deadenylation, translation, and decay of a wide spectrum of target mRNAs. Their discovery adds a new layer to the mechanisms of control of gene expression, impacting a broad range of biological processes. Some miRNAs have been shown to have direct anti-viral effects.

We have previously observed and validated that the fish-specific miRNAs, miR-462 and miR-731, were among the most highly expressed miRNAs in rainbow trout liver following *Viral hemorrhagic septicemia virus* (VHSV) infection. These miRNAs were also up regulated in the liver and muscle (vaccination site) of fish vaccinated with a DNA vaccine expressing the VHSV glycoprotein gene. Recent studies further indicate that the expression of these miRNAs is induced by interferons.

In order to analyze if miRNA-462 and miRNA-731 have any anti-viral effects, we designed inhibitory synthetic oligonucleotides called antagomiRs or anti-miRNAs. These saline-formulated 2'-O-methylated Locked Nucleic Acid (LNA)-based antagomiRs were injected intraperitoneally into rainbow trout fingerlings followed by exposure of the fish to VHSV. Development of disease and levels of infection will be analyzed and compared to data from fish treated with control miRNAs.

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Identification and expression of heat-shock protein 70 and ferritin in embryos of the calanoid copepod A*cartia tonsa* during transition between subitaneous and quiescent state

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The neritic calanoid copepod, Acartia tonsa (Dana), is capable of producing resting eggs to survive adverse environmental conditions which characterize environmental fluctuating estuarine systems. Subitaneous eggs hatch normally within a couple of days after spawning, while the resting state, quiescence, is a response of subitaneous eggs to a stressful environment.

Although physiological changes associated with this study, we investigate the molecular response associated with transition between subitaneous and quiescence. Two stress related proteins, ferritin and hsp70, were cloned from *A. tonsa* and their expression determined in embryos of *A. tonsa* during transition phases.

Expression of hsp70 remained low during quiescence despite the environmental stress. On the other hand, ferritin expression exhibited a strong increase from 72 hours till 120 hours of quiescence and then declined. After two weeks quiescence, development was recovered by the addition of oxygen and a temperature of 17 °C. The expression of both genes increased dramatically during the recovery phase toward hatching with an observed maximum after 96 hours of a 64 fold change for ferritin and a 257 fold change for hsp70 compared to 1 hour after inducing recovery.

This suggests that ferritin is a protein needed when embryos of *A. tonsa* enters quiescence. Both ferritin and hsp70 is needed during recovery from quiescent to subitaneous state.

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Amoebic gill disease

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Amoebic gill disease (AGD) is a condition caused by *Neoparamoeba perurans* affecting some species of cultured marine fish worldwide. Amoebic gill disease (AGD) is the most serious health problem of farmed Atlantic salmon in Tasmania. The only commercially available treatment is freshwater bathing. AGD has been reliably diagnosed with histological examination of gills although complementary methods such as *in situ* hybridization (ISH) and polymerase chain reaction (PCR) are required to confirm the presence of *Neoparamoeba perurans*. As molecular techniques are becoming more prevalent for pathogen identification, there is a need to adapt specimen collection and preservation so that both histology and molecular biology can be used to diagnose the same sample. We evaluated suitability of five different fixatives for both histology and molecular detection. Additionally, we evaluated the use of punched arrays for immunohistochemistry of fish gills. These methods will be applied in future AGD research.

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Immune regulating Es-products in parasitic nematodes

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Excretory/secretory (ES) products are molecules including various proteins produced by parasitic nematodes including larval *A. simplex* which is occurring in numerous marine fish hosts. The function of these substances and their effect on host physiology has not been fully described. The present work elucidates the effect of ES substances on the fish immune system by measuring immune gene expression in spleen and liver of rainbow trout (*Oncorhynchus mykiss*) injected intraperitoneally with ES products isolated from *A. simplex* third stage larvae. The overall gene expression profile of exposed fish showed a generalized down-regulation of the immune genes tested, suggesting a role of ES proteins in minimizing the immune reaction of rainbow trout against invading nematodes. We also tested the enzymatic activity of the ES proteins and found that lipase, esterase lipase, valine and cysteine arylamidases, naphthol-AS-BI-phosphohydrolase and α-galactosidase activities were present in the ES solution. This type of hydrolytic enzyme activity may play a role in nematode penetration of host tissue. Based on the notion that *A. simplex* ES-proteins may have an immune-depressive effect, it could also be suggested that worm enzymes directly target host immune effector molecules which would add to the decreased host immune responses and increased worm survival.

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Searching for vaccine candidates against salmon louse (*Lepeophtheirus salmonis*)

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The salmon louse (*Lepeophtheirus salmonis*) is a major problem in wild and farmed Atlantic salmon, causing mortality and serious economic losses. Currently, control of the parasite principally relies on chemical treatments, but this practice comes with several problems mainly related to resistance development and environmental effects. Alternative solutions, such as vaccination, are hence urgently needed. This strategy however, requires that suitable parasite antigens can be identified and used in the vaccines. In the present study, we show the preliminary results from a search for antigens in the salmon louse. Our strategy is to look for 'weak points' in the digestive system of the parasite. The salmon louse is a blood/mucus feeder and, hence, needs mechanisms to protect itself from harmful substances in the ingested host blood/mucus, such as immunoglobulins and complement factors. Using parasite survival and haemolytic assays, we have detected possible inhibitors of fish immune factors. Parasite survival assays showed that lice can survive up to 30 hours in diluted fish serum, and the haemolytic assays (fish plasma + rabbit erythrocytes + parasite proteins) showed that parasite molecules are able to inhibit erythrocyte lysis, likely by inhibiting the alternative pathway of the complement system. We used a technique called 'phage display' to try to isolate/characterize those molecules that protect the parasite. Using this technique, we have identified a salmon louse protein that binds to plates coated with salmon complement factor C3. Sequence analysis of this candidate has been completed (molecular and protein characterization). *In situ* hybridisation analysis showed that the protein is expressed in cells of the subcuticular tissue, intestinal wall and in some glands of the louse legs. Functional analyses of the candidate have been performed via *in vivo* RNAi assay (post-transcriptional gene silencing). The preliminary RNAi results indicate that louse survival and egg production were negatively affected in treated lice.

Funding: Norwegian Research Council (PrevenT), Alfonso Martín Escudero Foundation (FAME).

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Occurrence of salmon lice (*Lepeophtheirus salmonis*; Krøyer, 1837) in Danish wild Atlantic salmon (*Salmo salar*)

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Lepeophtheirus salmonis is an ectoparasitic copepod on salmonid fish in the Northern hemisphere, causing great economic losses to the Atlantic salmon (*Salmo salar*) farming industry. The pathology is due to feeding on mucus, epidermis and blood resulting in severe skin damages of the fish. In areas with intensive salmon farming, the salmon louse may further constitute a serious threat to wild populations of salmon and sea trout. In this study, a total of 62 salmon lice collected from wild Atlantic salmon in the River Skjern, Denmark, were examined and identified by morphometric means and by PCR. All lice were identified as *L. salmonis* based on their morphology. Further confirmation was provided by PCR of 19 lice which all had identical sequences and were identical to previously reported *L. salmonis*. In addition, the occurrence of hyperparasites was recorded and eggs of the monogenean worm, *Udonella caligus* were found on 11 % of the lice. The species were further confirmed by PCR providing sequences identical to previously reported *U. caligus*. No signs of intracellular pathogens, such as the microsporidian *Paranucleospora theridion* (*Desmozoon lepeophtherii*) were visualised microscopically or detected by PCR. This is the first study in recent years confirming the presence of *L. salmonis* in Danish rivers and suggests that sea lice might be more prevalent in Danish waters than first assumed. Alongside governmental plans for expanding salmonid production in Denmark, further studies framing potential lice threats to wild and cultured salmonid stocks in Denmark should be implemented.

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Knowing the weapons of your opponents

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Several groups of Crustacea parasitize fish, but the two groups with the highest impact are by far Caligid Copepoda and Branchiura, as they can cause extensive damage to the fish. The mouthparts of these crustacean ectoparasites are the "business end" of the animal, causing the actual physical damage to the integument of the fish host. Thus, to better understand the damages and their possible extents and causes, a detailed knowledge of the "weaponry" of the crustacean parasites is needed. In general, the mouthparts of these two groups are apparently fairly similar, in being tube-shaped, muscular and equipped with ripping mandibles, but in fact only relatively little is known about the detailed structures involved, and only older studies are available. In addition, both Branchiura and Caligid Copepoda have additional attachment and accessory feeding structures (hooks, spines etc), and there has been considerable debate and a widespread misunderstanding of their biological roles. In this study, I aim to clarify and give detailed illustrations of the complex structures of the mouth cones as well as the accessory structires from the Caligidae and Branchiura, based on my own Scanning Electron- and light microscopic investigations, as well as providing a summary of what is known (and not known!) about their integument damage capabilities.

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Serum Amyloid A Gene expression and immunohistochemical localization in rainbow trout, *Oncorhynchus mykiss*, infected by *Yersinia ruckeri*

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Serum amyloid A (SAA) is an integral part of the innate immune response in general and in particular the acute phase response. SAA belongs to a highly conserved group of apolipoproteins reported from different groups of organisms such as mammals, birds, fish and even invertebrates.

The present study was undertaken to elucidate the role of SAA protein in the innate immune response of rainbow trout. For this purpose a monoclonal antibody was raised against a recombinant peptide of rainbow trout SAA. The antibody was characterized using Western blot, immunohistochemistry and ELISA techniques. SAA was found to be associated with high density lipoprotein (HDL) which complicated band identification in Western blot, but delipidization of the SAA-HDL isolate, using a solvent extraction method, highly increased the quality of reaction in the Western blot. Inhibition ELISA indicated the presence of SAA in serum and tissues (head kidney, liver and spleen) of rainbow trout. Rainbow trout fry (87 days post hatch) infected with *Yersinia ruckeri* showed a significant up-regulation of the SAA gene at 72 h post infection with further increase at 96 h post infection. Non-significant up-regulations were seen at earlier time points *i.e.* 4 and 24 h. A weak staining with the monoclonal antibody was seen at 4 h post infection which increased in intensity during the course of infection *i.e.* 24, 72 and 96 h post infection. The expression pattern of SAA in the infected fry, analysed by qPCR, significantly correlated with the results obtained by immunohistochemical methods. From the present study it can be concluded that the SAA may act as an acute phase protein in rainbow trout and its expression increases significantly during the course of infection.

Keywords: SAA, rainbow trout, acute phase protein, innate immune response, monoclonal antibody

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Immune stimulating compounds and their use in fish production

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Preparations of β-glucan are widely used as immunostimulatory feed additives in food fish production. β-Glucans form a diverse group of linear and branched polysaccharides produced as structural and storage components by bacteria, fungi, algae, lichens and plants. Since β-glucans are absent from metazoans, these polysaccharide structures are considered as classical pathogen associated molecular patterns (PAMPs) recognized by pattern recognition receptors (PRRs) of the innate immune system. However, the ability and potential of a β-glucan to stimulate the immune system appear to be dependent on a multitude of variables, e.g. linkage type, linearity, degree of polymerization, branching frequency, solubility, etc., which all are determined by the source of origin and may be altered during extraction procedures and further physical and chemical treatments. Despite a considerable body of published research on the immunostimulatory effects of β-glucans in both fish and mammals, no consensus has been achieved regarding the basic physico-chemical β-glucan features required for immunostimulation. This is most probably explained by the extensive use of impure β-glucan preparations in research and the lack of essential information such as structure, solubility, source of origin and purity of the particular β-glucan in question. Imprecision of experimental design and insufficient information about these compounds hampers reproducibility of results and the development of an in-depth understanding of β-glucans as immunostimulants. This presentation will provide an overview of the complexity of β-glucans, receptors of the immune system recognizing these molecules, and the associated immunomodulatory effects observed. Finally, the potential and applicability of β-glucans as immunostimulators in fish production will be assessed.

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Zebra fish as a model for inflammatory bowel disease (IBD)

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Traditionally, the zebra fish (*Danio rerio*) has been used primarily as a vertebrate model system in developmental biology. The optical clarity of embryo and larvae permits visualization of cell-biological events *in vivo* and, owing to the fecundity of the adult fish, a high number of experimental animals can be obtained. Further, transgenic and knock-out lines can be established relatively easily. Based on these favourable traits the zebra fish has emerged as a promising animal model in the field of human disease studies. Researchers working with a diverse array of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and type 1 diabetes have recently been including this model in their studies.

This PhD project will investigate the potential of the zebra fish as a model for inflammatory bowel disease (IBD), a human autoimmune disorder. IBD is a debilitating disease resulting from a discordant relationship between the host and the intestinal bacteria. Genetic susceptibility also plays a major role. Two IBD models will be produced, a chemical and a molecular: IBD will be induced in the former through intrarectal injection of oxazolone whereas the latter model is achieved by producing IL-10 knock-out fish and subsequently accelerating the disease progress with piroxicam. Once established, the models will be compared with already existing rodent models and general aspects of the human disease. Histology, immunohistochemistry, electron microscopy and quantitative real-time PCR (qPCR) will be used to characterize the models. Furthermore, the gut microbiota will be sampled as the composition is known to be different in diseased patients. The effect of antibiotics and a variety of conventional anti-inflammatory drugs are also expected to be investigated. By performing both chemical and biological treatment experiments on several different zebra fish IBD models, a more integrated overview of the models and their potential translational impact is hopefully achieved.

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Cellular immune reactions in rainbow trout

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The main function of any immune system is to keep homeostasis when invaded by pathogens. Prior to successful elimination of invaders it is crucial to distinguish self from non-self. Pathogens, foreign and altered cells can be identified by immune cells through expressed pathogen associated molecular patterns (PAMPS), by presenting foreign non-self peptides of intracellular (through MHC class I - *e.g.* virus infected target cells) or extracellular (through MHC class II - *e.g.* from bacteria) origin, and when their MHC class I is missing (*e.g.* when down-regulated during infection or different in grafted cells).

Although grafting does not play a role in fish health it provides nice models for studying general mechanisms of immune response. In order to eliminate invaders directly or by destroying their cellular replication basis (*e.g.* virus infected cells) specialized immune cells of the innate and adaptive responses appeared during evolution. The first line of cell-mediated defence is represented by macrophages, neutrophils and natural killer (NK) cells. These innate mechanisms evolved early in evolution and are thus well developed in bony fish. At the adaptive level, professional antigen presenting cells display peptides loaded into MHC class II molecules to the T cell receptor (TCR) of T helper (Th) cells. Depending on the type of antigen Th cells produce cytokines that control further immune responses. Cytotoxic T lymphocytes (CTL) kill cells harbouring intracellular pathogens by binding of their TCR to an MHC class I/peptide complex on the infected host cells.

During the recent years, genes encoding MHC molecules, TCR, its co-receptors CD4 and CD8 on Th cells and CTLs (respectively) have been cloned in several fish species and a few antibodies were developed to study protein expression in a morphological and functional context. While cell-mediated immune functions such as phagocytosis are relatively easy to study in most fish species functional assays for innate and adaptive CMC were only developed in a few species. This presentation reflects some of our results in the field of T cell immune responses.

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Characterization of peptides loaded into mhc class i in IHNV infection of rainbow trout (*Oncorhynchus mykiss*)

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Cell mediated immunity is an important defence mechanism against virus infections in higher vertebrates. Also in fish, specific cell mediated cytotoxicity against virus infected cells has been demonstrated by functional assays. Moreover, the existence of cytotoxic T lymphocytes (CTL) has been confirmed by gene expression profiling of specific cytotoxic effectors cells, and more recently with antibodies against their population specific surface marker CD8. Binding of short antigenic peptides derived from processed viral proteins to major histocompatibility complex (MHC) class I molecules is essential for an adequate antigen presentation on the surface of infected host cells. Those peptides are then specifically recognized by the T cell receptor (TCR) of CTLs in an MHC class I restricted fashion triggering clonal expansion of antigen-specific CTLs. The identification of antigenic peptides as potential TCR epitopes is crucial for the understanding of immune responses and for vaccine development.

In order to predict potential viral peptides that are involved in MHC class I restricted presentation during infectious haematopoietic necrosis (IHN) we have used a NetMHCpan computational prediction system. After uploading the full length protein sequence of the rainbow trout MHC class I allele UBA*150101, as well as of the glycoprotein of the IHN virus, several nonapeptides of different binding intensities were predicted. In parallel, the extracellular heavy chain domain of UBA*150101 and of the β2-microglobulin (β2m) were expressed in *E. coli*. The purified recombinant proteins were then incubated in the presence of the predicted synthetic nonapeptides and tested for the formation of properly refolded monomers by size exclusion chromatography. Those peptides that have induced proper complexing of MHC class I and β2m will be employed for preparation of fluorescent labelled MHC class I tetramers. Those tetramers will then be used for the recognition, isolation and characterization of antigen-specific T cells induced by viral infection or vaccination.

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Selective breeding for liveability in a commercial breeding programme

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Liveability can be influenced by genetic factors in 3 different ways:

1. Chromosome defects
2. Single genes with major effects
3. Poly-genetic factors, many genes with small effects.

Chromosome defects are often lethal or give deformations which can by semi-lethal.

Single genes with major effects, also called resistant genes, can have a major effect on resistances to some diseases.

Liveability is very complex and organisms well buffered with multiple defence mechanisms have a better chance to survive stress and disease attack.

The genotype values for liveability have a normal distribution like other quantitative traits like growth and body size but the phenotype has only two values, dead or live.

There are three different ways to select for liveability in a commercial breeding programme.

A certain level of the families with highest mortality can be culled, and thus probably reduce the frequencies of chromosome defects and genes with major negative effects.

Many genes with small effects on liveability result in a genotypic values resembling a normal distribution. The phenotype is only seen in two discrete classes -dead or live. These values can be used to estimate a breeding value for liveability of their live family members, and then included in a multi trait selection process.

Immune response can be used as selection criteria for a general liveability. It can be used even in a non-pedigreed population. The results have a better distribution than the binary mortality results, and it can be simpler and cheaper than a challenges test.

The methods will be illustrated by field data from poultry and fur animal breeding programmes.

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Is the intestinal microbiota in rainbow trout (O*ncorhynchus mykiss*) influenced by diet type and challenge by *Yersinia ruckeri*?

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In recent years it has become more and more evident that the bacterial flora in the gut of warm-blooded animals modulates physiological processes and the immunological status of the host. Besides effects on growth parameters, commensal intestinal bacteria balance the immune system and prevent colonization of pathogenic bacteria. The question is if the gut microbiota is also important in lower vertebrates such as fish? And does it play a role in connection to pathogenic challenge? To examine these questions rainbow trout fry were fed two different diets of either a marine or vegetable origin directly after first feeding. At a size of about four gram the fish were bath challenged by *Yersinia ruckeri* and intestines were then sampled 5 and 18 days post challenge for subsequent metagenomic and immunological examinations. Next-generation sequencing was applied for the metagenomic studies using the Illumina HiSeq platform. The results clearly showed two different microbial patterns in the intestines dependent on the diet type. Control fish fed a marine based diet overall had a higher amount of proteobacteria, while high amount of reads belonging to phylum Firmicutes dominated in the intestines of vegetable fed fish. Several genera within the order Lactobacillales belonged to the many reads from Firmicutes. In challenged fish with a high load of reads from genus *Yersinia* there was a significantly lower amount of reads from the order Burkholderiales. Further, these fish further clustered separately when analyzing the bacterial community on a PCA plot. The immunological examinations using RT-qPCR showed no different expression patterns between the diet groups in control fish, but the response was very different in connection to challenge. Here, the general pattern was a pro-inflammatory response in the intestine of marine fed fish challenged with *Yersinia ruckeri*, while several immune genes were down-regulated in vegetable fed fish. Overall, the results indicate that the gut microbiota in rainbow trout is highly plastic according to the type of diet and does further seem to be involved in the immunological response in connection to pathogenic challenge.

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Comparative analysis of immune cells of the primary and secondary vascular system in rainbow trout *Oncorhynchus mykiss* (Walbaum)

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The vascular system in fish is divided into two components, the blood circulation or the primary vascular systems (PVS) and a secondary vascular system (SVS), which is a vessel system referred to by earlier authors as 'lymphatics'. The SVS forms capillary beds situated in the outer surfaces of the fish, such as the skin, gills, mouth and pharynx but appears to be absent in regions of the mesenteric and renal tissues, where the lymphatic system is normally found in mammals. These anatomical characteristics and occasional presence of red blood cells in SVS call for a reassessment of whether this vascular compartment has a lymphatic function as seen in mammals.

The present work partly characterized and compared immune cells in the primary and secondary vascular systems of the rainbow trout *Oncorhynchus mykiss* (Walbaum). Cell counts were performed from fluid withdrawn from PVS and SVS. Measurements showed that the absolute density of leukocytes in the PVS (3.8x107 cells mL-1) was ten-fold higher compared with the SVS (3.1x106 cells mL-1) whereas the relative share of leukocytes in relation to the total blood cell number was substantially higher in SVS (66%) than in PVS (4-5%). Immunocytochemistry of fixed cells from the PVS and the SVS indicated a different distribution of cell types. Lymphocytes (such as IgT positive cells and CD8+-positive putative T-cells) occurred at a much higher frequency in the SVS whereas MHCII positive cells were slightly more prevalent in PVS.

These findings suggest that although SVS have diverse physiological roles it may also play an immunologically specialized role *e.g.* in mucosal immunity.

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Detection and quantification of *Flavobacterium psychrophilum* specific bacteriophages in rainbow trout upon different administration methods: Implications for disease control in aquaculture

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Flavobacterium psychrophilum is the pathogen causing the disease rainbow trout fry syndrome (RTFS), which has important implications for aquaculture production and trade worldwide. RTFS can be treated by antibiotic administration, but with the increasing problem of antibiotic resistant bacteria, the use of lytic bacteriophages is a promising alternative approach to disease control in aquaculture.

Bacteriophage control of bacterial infections depends on efficient delivery of the phages to the infected organs, and in this study we therefore examined the occurrence and persistence of phages in the internal organs in rainbow trout, following different administration methods. Three phage administration methods using phage FpV-9 were used: phage bath, oral administration of phage-suspension directly into the stomach and feeding with phage-coated feed pellets.

Phages were detected in all the four examined organs (intestine, brain, spleen and liver) with all three administration methods, demonstrating that the phages are capable of passing the intestinal wall and entering the bloodstream. The highest phage concentration was found in the intestine where a maximum of $3x10^{10}$ phages g^{-1} was obtained after oral administration of phage-suspension, but also phage addition via phage-coated feed pellets resulted in high phage titers ($5x10^{6}$ phages g^{-1} intestine). The concentration of phages in the spleen was 100 fold lower than in the intestine, suggesting a large phage decay during transport to the inner organs. These results provide the basis for future phage treatment of RTFS.

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Vaccination strategies in salmonid aquaculture

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Many aquaculture vaccines have been successfully used to minimize risks of disease outbreaks on fish farms. Most vaccines are applied by injection; however those which are required in early life stages are usually applied by immersion. Oral vaccination has a lot of advantages but is not commonly used by the industry. Vaccine efficacy is usually tested through challenge experiments, which are time consuming and require many controls and as a result high numbers of experimental fish. We propose the use of transcriptional biosignature as an alternative which would improve fish welfare and allow faster testing of vaccines with a direct application for aquaculture industry. Yersiniosis is a bacterial disease affecting Atlantic salmon in Tasmania during hatchery stage and after transfer to the sea. The disease is caused by *Yersinia ruckeri*. While there are commercial vaccines available, outbreaks still occur at least partly due to the presence of carriers. Using cDNA microarray we identified the expression of 6 genes in response to infection and 4 genes associated with the protective host response to yersiniosis. A transcriptional biosignature consisting of predominantly immune-relevant genes (14 up and 3 down-regulated) in the gills of Atlantic salmon after immersion vaccination and before bacterial challenge was identified. This biosignature may be used as a surrogate of protection and therefore as a predictor of vaccine success against yersiniosis. We have tested an effect of a novel inactivation method of *Y. ruckeri* on vaccine efficacy and showed that the transcriptional signature was a predictor of protection, thus confirming our previous results.

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Parasites as biological tags in fish population studies

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The use of parasites as biological tags in fish population studies is reviewed. The method dates from 1939, since when it has been used with increasing frequency. The history of parasite tagging is presented, the changing criteria and guidelines applied to the selection of tag parasites are given, and landmark publications are highlighted. The advantages and disadvantages of parasite tags compared with other methods used in fish population studies, such as host genetics and mechanical tagging, are discussed. Recent trends and developments such as the use of molecular methods and the application of multivariate analyses are described. Parasite tags are most effective when used in combination with other tagging methods in multidisciplinary projects and some successful recent studies are described to illustrate this point. Finally, the potential of parasite tags for stock identification of South African sardines is examined.

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Infection of North Sea cod (*Gadus morhua* L.) postlarvae and juveniles with the parasites *Hysterothylacium aduncum* Rudolphi and *Caligus* sp.

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Parasitic infections of individual juvenile and adult Atlantic cod (*Gadus morhua* L.) have been well studied for decades, but infections of early life stages and the impact of parasitism on population level have been less well elucidated. It is generally assumed that early developmental stages of fish are more vulnerable to infection compared to older age groups, but merely few investigations on parasitic infections in young cod are available. We have therefore performed a parasitological investigation of a total of 3361 specimens of Atlantic cod post larvae and juveniles sampled from the North Sea in 1992, 1993, 1994, 1999 and 2001. Two metazoan parasites *Caligus* sp. and *Hysterothylacium aduncum* (Rudolphi) were found at relatively high frequencies. *Caligus* sp. showed a higher infection level in 1992 compared to the following years, whereas the prevalence of *H. aduncum* increased from 1992 to 2001. It was indicated that these young stages of cod were not able to tolerate high parasite burdens which suggests that survival may be affected by a high infection pressure. We also analysed if infection with *H. aduncum* would influence growth of cod post-larvae. This was done by comparing the body size of infected (1-2 parasites per fish) and uninfected fish sizes in various age groups. Ageing was performed by otolith readings, and it was indicated that cod younger than 44 days were negatively affected by infection whereas cod older than 44 days tolerated this low parasite burden.

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Immune response of rainbow trout larvae against *Ichthyophthirius multifiliis*

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From fertilization to the time of hatching the rainbow trout embryo is well protected by the enclosing chorion. When the larva hatches it is directly exposed to the surrounding environment and it has to be able to resist or overcome infection by disease causing organisms in the freshwater aquatic environment. The immune system of rainbow trout larvae is not fully developed when the larvae hatch from the egg but the immune defense during this early developmental phase is largely unknown. The protozoan skin parasite *Ichthyophthirius multifiliis* is a common parasite in fish and immunological mature rainbow trout are able to respond against infection. Here, we investigated the ability of 10 days post-hatch (84 degree days) larvae to respond towards infection with *I. multifiliis*. Quantitative RT-PCR (qPCR) was used to analyze the expression of the immunologically relevant genes IL-1β, IL-8, IL-6, TNF-α, iNOS, SAA, cathelicidin-2, hepcidin, IL-10, IL-22, IgM and IgT. In addition, a panel of 5 monoclonal antibodies was used to investigate the presence and localization of the proteins CD8, SAA, MHCII, IgM and IgT in the larvae. Samples for qPCR were taken at 3, 6, 12, 24, 48 and 72 h post-infection (p.i.) and at 72 h p.i. larvae for antibody staining were sampled. At 3 h p.i. IL-1β was up-regulated as the first of the studied genes. Following the first IL-1β expression, IL-8 and cathelicidin-2 were up-regulated at 6 h p.i. At later time points, up-regulation was seen for TNF-α, hepcidin, IL-6, iNOS and SAA. Monoclonal antibody staining of CD8 and MHCII was seen in the thymus of both infected and non-infected larvae. Also, staining of SAA and IgT was seen in the infected larvae. The present investigations have shown that 10 days (84 degree days) post-hatch larvae are able to mount an immune response to infection with *I. multifiliis* and regulate the expression of cytokines, chemokines and acute phase proteins.

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Antiviral immunity in fish – functional analysis using DNA vaccination as a tool

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In fish, DNA vaccines encoding the glycoproteins (G proteins) of the salmonid rhabdoviruses VHSV and IHNV have proved very efficient under experimental conditions. Nano-gram amounts of plasmid DNA can induce long-lasting protective immunity when delivered by intramuscular injection in rainbow trout fingerlings. Vaccination of fish at an early stage appears advantageous, since larger fish require higher doses of vaccine to be protected. Even in fish with an average size of 0.5 g at the time of vaccination, good protection can be obtained. Interestingly, immunity is established already a few days after vaccination and cross-challenge experiments have revealed that protection in the early phase is non-specific. Later on, protection becomes very specific in terms of virus species. The protection in the early non-specific phase is related to interferon induced defence mechanisms whereas specific antibodies and cellular components both play a role in the long lasting protection. The similarity of the functional immune response profile to that induced by a natural virus infection is striking and is most likely one of the major reasons for the efficacy of the rhabdovirus DNA vaccines. Although other elements like CpG motifs in the plasmid backbone sequence might play a role, the viral G protein appears to have an inherent ability to stimulate innate immune mechanisms by receptors and pathways that still remain to be characterized in detail. Immunity to VHS in rainbow trout can be induced by DNA vaccination across a temperature range of at least 5-15°C. Interestingly, the initial non-specific phase is significantly prolonged at lower temperatures, hereby ensuring protection despite a slow activation of adaptive mechanisms. Expression of the rhabdovirus G protein on the surface of transfected muscle cells induces a histologically visible local inflammatory reaction with higher doses of VHSV G DNA vaccine. Cell surface expression may be important for a proper activation of the fish immune system, since blocking of the intracellular trafficking of the expressed glycoprotein G-gene interferes with protection. It may be anticipated that the viral G protein acts like a PAMP (pathogen associated molecular pattern), but it remains to be determined which PRRs (pattern recognition receptors) that may be involved in the recognition of the G protein. Recent data from DNA vaccination trials with variant forms of the G protein gene suggest that the structural requirements for antigenicity are different from the requirements for immunogenicity.

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Biology of Map-1, a novel regulatory member of the complement system that attenuates myocardial ischemic reperfusion injuries and inhibits arterial thrombogenesis *in vivo*

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The lectin complement pathway (LCP) is characterized by circulating complexes consisting of mannose-binding lectin or ficolins in association with serine proteases called MASPs. We have recently described a novel LCP member named MBL/Ficolin associated protein 1 (MAP-1) derived from the *MASP1* gene through differential splicing. We have shown that MAP-1 is highly expressed in myocardial and skeletal muscle tissues reflecting a different expression profile compared to the MASPs. Furthermore, we found high affinity interactions between MAP-1 and MBL/ficolins in the 5 nM range and observed a strong inhibitory effect on the complement activation of C4, C3 and the terminal complement complex.

We determined the crystal structure of MAP-1 illustrating an elongated dimer molecule of 146 Å length that includes 12 calcium interaction sites and two N-linked $GlcNAc_2Man_3$ glycans at positions N49 and N178. The structure also reflects the flexible junction region between the CUB_2/SCR_1 domains as well as the unique 17 C-terminal residues that appear to be disordered in the crystal.

Cross-talk between the coagulation and complement system have been reported previously. Coagulation disorders and reperfusion of ischemic myocardium are major causes of morbidity and mortality. We addressed the possible role of MAP-1 *in vivo* in relation to coagulation, ischemia/reperfusion and maintenance of tissue homeostasis. We found that MAP-1 attenuated myocardial ischemia/reperfusion injuries and thrombogenesis in two different mice models and showed that MAP-1 preserved cardiac function, decreased infarct sizes, inhibited the tissue deposition of complement and prevented thrombogenesis *in vivo*.

Taken together, our results suggest that the natural, endogenous complement regulator, MAP-1, effectively inhibits the lectin complement pathway activation *in vivo*. And that MAP-1, when administered at pharmacologic doses, represents a novel therapeutic approach for diseases involving coagulopathy and ischemia/reperfusion events.

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Three-dimensional imaging analysis of *Yersinia ruckeri* infected rainbow trout (*Oncorhynchus mykiss*) gills by optical projection tomography

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Optical projection tomography (OPT) is a new tool for three-dimensional (3D) imaging of small tissues or embryos, based on multi-angle recording of internal fluorescent signals using intact whole mount tissue or fish. To understand the route of infection, gills of *Y. ruckeri* infected rainbow trout were labeled with fluorescent antibody and visualized in 3D by the OPT scanner. Rainbow trout were infected with *Y. ruckeri* O1 biotype 1 (1 x 10^9 cells/ml) for 1 hour at 18 °C, and then transferred to clean water. Three fish were sampled at 12 different time points and fixed in 4% PFA. The gills were incubated whole with rabbit anti-*Y. ruckeri* polyclonal antibody and Alexa Fluor®594 conjugated goat anti-rabbit IgG. After embedding in 1% low melting point agarose, specimens were dehydrated in 100% methanol and cleared in BABB (benzyl alcohol: benzyl benzoate) for OPT scanning. 3D imaging results showed that *Y. ruckeri* were observed in connection with the primary gill filaments, at the base of gill arches. In contrast, no bacteria were found in control fish gills. More detailed analyses are being processed.

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Seahorse symphony – Why fish never cease to inspire

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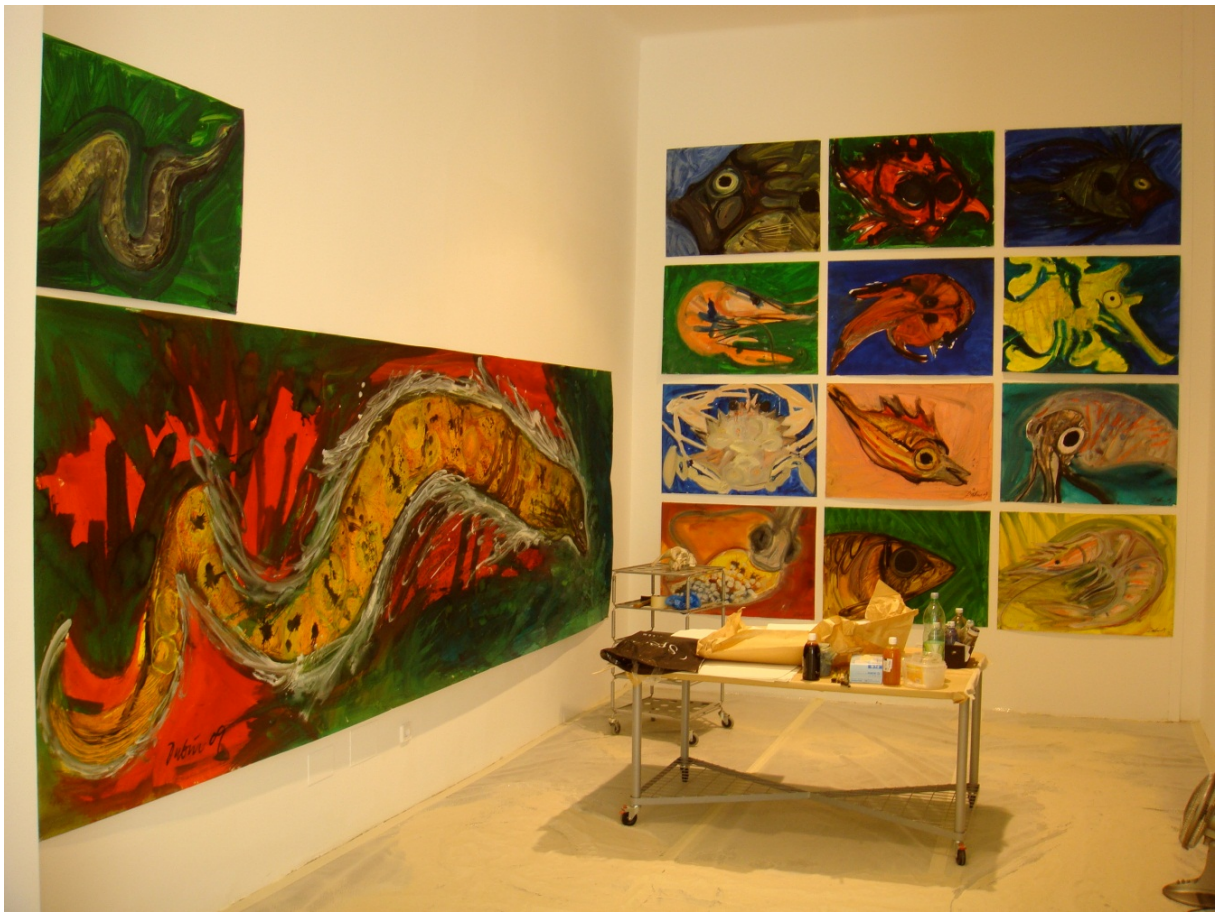
Painter and artist Maria Dubin is currently working on a project called “Seahorse Symphony”. This fascinating fish is a never ending inspiration for Maria, not only when decorating living blocks in Måløv, Denmark, but also when she as the artistic leader creates new cities in Serbia. Today Maria will bind science and art together in a sea horse symphony and show examples of her work.



Organised in collaboration with the

KVL Art Association

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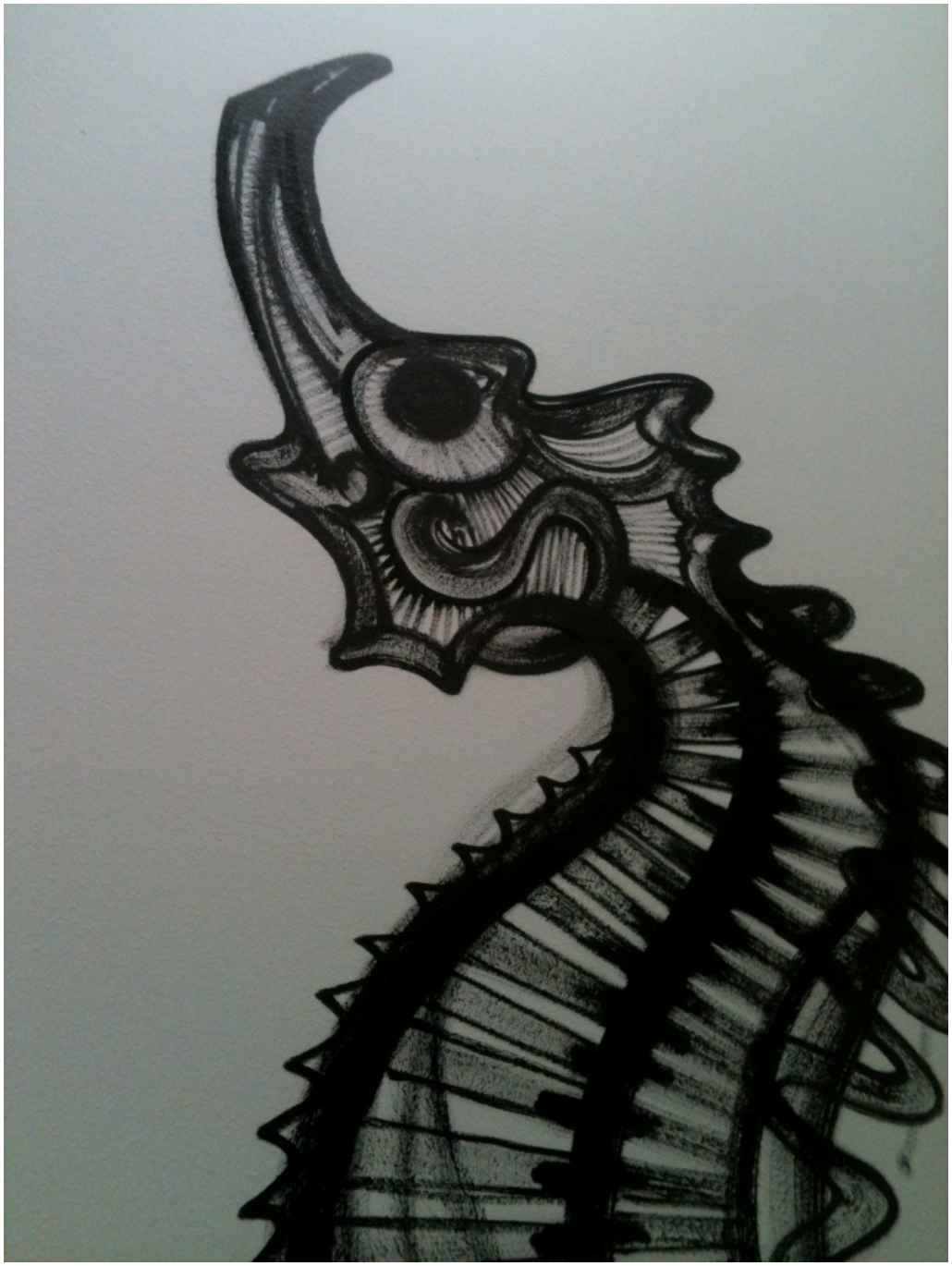

Illustrations by Maria Dubin