Genetics of VHSV in Europe

Cieslak, M.; Baud, M.; Diserens, N.; Engelsma, M.; Haenen, O.; Mousakhani, S.; Olesen, Niels Jørgen; Panzarin, V.; Skall, H. F.; Wahli, T.; Schütze, H.

Published in:
17th International Conference on Diseases of Fish And Shellfish

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

DISCLAIMER: The organizer takes no responsibility for any of the content stated in the abstracts. The abstract book contains abstracts as provided by their authors.
No editing has been done except for spelling corrections.

Published for:
European Association of Fish Pathologists, 2015
Printed and bound in Las Palmas

Picture front cover provide by Auditorium Alfredo Kraus

BOOK OF ABSTRACTS

Contents

KEYNOTES

ORAL PRESENTATIONS

POSTER PRESENTATIONS

Conference keynote

CHALLENGING THE DOGMA SURROUNDING THE STUDY OF BACTERIAL FISH DISEASES

B. AUSTIN

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

Since the late nineteenth century, the acquisition of pure bacterial cultures has been central to all facets of microbiology. Fish pathology is no exception insofar as the goal of disease diagnostics and research is the acquisition of pure cultures of the pathogen. The basic premise is that pathological material may be inoculated onto a solid (usually gelled with agar) or into a liquid medium with incubation for a pre-determined interval when individual cells of the pathogen will be cloned into dense culture growth, which will then be subjected to further study. However, the range of media used by fish bacteriologists is restricted, and often centres on tryptone soya agar/broth, brain heart infusion agar/broth and/or marine equivalents. Moreover, the incubation regimes may have little relevance to the growth conditions of the fish. However, the desired outcome is the presence of dense virtually pure growth, which is taken as indicative of recovery of the pathogen. Unfortunately at best, a snap shot of the disease is obtained, and it may not be possible to decide if only one organism instigated the infection, and then contributed to the development of overt disease signs. It is unlikely that culturing on a single occasion would identify microbial population succession within a disease cycle. Also, conventional techniques are unlikely to recognise when two or more discrete organisms working synergistically to produce a single pathology. This situation has been observed with ulcerative conditions in cyprinids when *Aeromonas salmonicida* and *A. hydrophila/ A. sobria* may be involved together, with the former instigating infection, and the latter leading to the developing of large ulcers. Certainly, it is realized that not all cells will multiply sufficiently to produce visible colonies. Some cells produce micro-colonies that are invisible to the naked eye. Moreover, the proportion of culturable cells that produce visible growth will vary according to the species and the state of the cells – are they actively growing or comparatively inactive? The latter have a poorer rate of recovery in terms of culturability. The next premise is that an individual colony is derived from multiplication of a single cell. Yet, it is realized that cells in close proximity to each other may multiply and come together to produce a single colony. Then, the resultant growth will most certainly be derived from more than one initial cell. This has greater relevance if the two initial cells are from two different species. Although it is generally assumed that streaking and re-streaking on fresh media will purify any culture, there is evidence for microbial consortia interacting to form what appear to be single pure cultures. Thus, seemingly pure cultures of purple-pigmented aquatic bacteria were recognized to contain cells of *A. salmonicida*. As so-called pure cultures underpin most of microbiology, it is relevant to understand that the culture does not necessarily contain clones of identical bacteria, but that there is a variation in the genetic potential of the component cells, i.e. the cells are not homogeneous. Certainly, many bacteria change rapidly upon culturing in the laboratory. Cells may become bigger and less active in the laboratory, i.e. genetic potential is lost. It is difficult to be sure if the changes reflect a loss of DNA or whether standard culturing methods select faster growing cells that are effectively not representative of the environment from which they were derived.

AQUATIC VERSUS NON-AQUATIC HERPESVIRUSES: SIMILARITIES AND DIFFERENCES

A. VANDERPLASSCHEN

Immunology-Vaccinology, Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

The order *Herpesvirales* encompasses viruses that share structural, genetic and biological properties. However, members of this order infect hosts ranging from molluscs to humans. It is currently divided into three phylogenetically related families. The *Herpesviridae* family encompasses viruses infecting mammals, birds or reptiles. It is by far the most important, in terms both of the number of its members and the volume of studies that have been devoted to them. The *Alloherpesviridae* family encompasses viruses infecting fish and amphibians. And finally, the *Malacoherpesviridae* family comprises viruses infecting molluscs. Over the last decade, an increasing number of studies have been devoted to alloherpesviruses and malacoherpesviruses. Scientific interest in these viruses tends to originate from their impact on wildlife, the economic losses they cause to the aquaculture industry, or their importance as fundamental research object. In this talk, we will review the similarities and the differences existing between herpesviruses infecting aquatic and non-aquatic animals.

WHAT'S NEW IN BIVALVE MOLLUSC PATHOLOGY? OVERVIEW OF ARTICLES PUBLISHED IN THE LAST TWO YEARS

A. VILLALBA

Centro de Investigacións Mariñas (CIMA), Consellería do Medio Rural e do Mar da Xunta de Galicia, 36620 Vilanova de Arousa, Spain

This presentation is an overview of articles on bivalve mollusc diseases in the last two years. Two journals have devoted special numbers to this subject, one on "Microcell Parasites of Molluscs" by *Diseases of Aquatic Organisms* and another one coming soon on "Pathogen and Diseases Processes in Marine Molluscs" by *Journal of Invertebrate Pathology*. Two items stand out for the number of papers, the infection of the Pacific oyster *Crassostrea gigas* with the Ostreid herpesvirus 1 (OsHV1) and the bivalve immune response, mostly through transcriptomic/proteomic changes associated with pathogens/diseases. Analysis of environmental samples with molecular diagnostic tools has allowed detecting pathogen prints in remote disease-free locations as well as unsuspected pathogen diversity. Regarding OsHV1, an outstanding research effort on a wide thematic variety has resulted in useful knowledge to fight this disease: geographical range, genetic variability and host range of the herpesvirus; viral genome and transcriptome; viral phylogeography; diagnostic tools; OsHV1 associated oyster mortality; genes involved in the oyster immune antiviral response, with candidate gene markers of oyster resistance; enhancement of oyster specific antiviral response providing protection against OsHV1; host (size, age, ploidy) and environmental factors influencing disease dynamics; influence of and effects on host physiology; effects of toxic algae on the virus-oyster interaction, influence of husbandry practices and design of management procedures to minimise mortality; and selective breeding, by which remarkable increase of oyster survival has been achieved. Gene expression of the scallop *Chlamys farreri* challenged with acute viral necrobiotic virus has also been addressed. Much attention has also been focused on bacteria, with description of new pathogenic species, frequently in hatchery context. Among vibrios, addressed issues include extracellular products, virulence factors, genetic variability, effects on host physiology, mortality in hatcheries of *C. gigas* and *Argopecten purpuratus*, autophagy as a host protecting way, and link between rapid evolution of resistance to local *Vibrio* spp. and invasive capacity of Pacific oysters; regarding brown ring disease, Manila clam genes and enzymes involved in immune response, population structure of *V. tapetis*, and a model of disease dynamics have been addressed. Other reports include the transcriptome of *Crassostrea virginica* challenged with the agent of the juvenile oyster disease; characterisation of haemolymph microbioma of *C. gigas*; new host, location and diagnosis procedure of *Nocardia crassostreae*; and managing procedures to fight bacteria, including the use of probiotic strains. Protists have also focused attention, mostly perkinsosis, bonamiosis, marteiliosis, and mikrocytosis but also QPX, dinoflagellates and others, addressing new hosts and locations, new species, taxonomy and phylogeny, diagnosis, life cycle, transmission ways, parasite transcriptome and proteome, associated mollusc mortality, influence of environmental factors, effects on host physiology, host genes involved in the immune response, molecular host-parasite interaction, drugs inhibiting parasites, and selective breeding to increase host resistance. Among various papers devoted to neoplasia, that reporting horizontal transmission of clonal cancer cells as the cause of leukaemia spreading in clam *Mya arenaria* populations stands out. Other subjects include pathogenic metazoans, climate change, health condition surveys of various bivalve species and health management.

ANIMAL BASED WELFARE INDICATORS: A BIOMEDICAL APPROACH TO FISH WELFARE. SOME COMMENTS ON RECENT CHANGES IN LEGISLATION ON ANIMAL TRIALS

P.J. MIDTLYNG[1, 2]

[1]*School of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway*
[2]*Aquamedic AS, Lörenskog, Norway*

Current controversies over how the welfare of production animals including farmed fish shall be judged, and also recent changes in European legislation on the use of animals for scientific purposes illustrate a highly politicised field in which animal rights positions are gaining more acceptance by legislators. This development is followed by an increase in the administrative requirements and -burden for those who keep fish for food production or for scientific research. To counteract the upcoming bureaucratic frenzy, this presentation advocates the concept of "risk-based controls" in fish welfare, a concept that has long since been implemented in the control of infectious diseases. Risk-based management means that monitoring and control efforts should be focussed on those experimental animals whose welfare is considered most at risk, while control resources should not be wasted on projects and procedures likely associated with negligible or minor welfare risk or consequences. Neither should interventions that are recommended nor even mandatory in good animal husbandry (e.g. vaccinations or marking for identification) be subjected to excessive administrative rules when used for the purpose of science or education. In order to "de-politicise" animal welfare this author advocates the development, validation and continuous improvement of animal-based welfare indicators. A recently completed Norwegian research project has attempted to develop a number of animal-based welfare indicators for farmed Atlantic salmon held in seawater cages. Based on a list of the salmon´s main physiological needs, indicators that are associated with the welfare relevant (lack of) fulfilment of these needs were selected. Through a scoring and weighting procedure applied to each and all of the indicators, two version of the Salmon Welfare Index Model (SWIM) were proposed. While SWIM 1.0 was designed for use by fish farmers, SWIM 2.0 was expanded by searching the literature for documented welfare indicators that could be used by fish health professionals. Selection criteria for the SWIM indicators were that they should be practical and measureable on salmon farms, and that each indicator could be divided into levels from good to poor welfare backed up by relevant scientific literature. Pros and cons of selected animal based indicators used in the SWIM models will be discussed.
The presentation will last not least discuss some recent views on the use of hormonal markers (e.g. cortisol and brain serotonin) as indicators of welfare relevant experiences of fish.

FULL-LENGTH SEQUENCING AND ANALYSIS OF 25 CYHV-3 SPECIMENS REVEALS ATYPICAL GENOMES WITH HIGH DIVERGENCE

S. HAMMOUMI[1], A. SANTIKA[2], Z. ZAINUN[2], T. VALLAEYS[3], E. BORZYM[4], S. BERGMANN[5], C. KLOPP[6], M. ENGELSMA[7], J.C. AVARRE[1]*

[1]*Institut de Recherche pour le Développement, Montpellier, France*
[2]*Main Center for Freshwater Aquaculture Development, Sukabumi, Indonesia*
[3]*Université de Montpellier, France*
[4]*National Veterinary Research Institute, Partyzantow, Poland*
[5]*Friedrich Loeffer Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems*, *Germany*
[6]*Plate-forme bioinformatique Genotoul-INRA, Castanet-Tolosan, France*
[7]*Central Veterinary Institute of Wageningen, Lelystad, The Netherlands*

Koi herpesvirus disease (KHVD) is an emerging disease that causes mass mortality in koi and common carp, *Cyprinus carpio* L. Its causative agent is Cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus (KHV). Although data on the pathogenesis of this deadly virus is relatively abundant in the literature, still little is known about its genomic diversity and about the molecular mechanisms that lead to such a high virulence. In this context, we developed a new strategy for sequencing full-length CyHV-3 genomes directly from tissues of infected fish. Total genomic DNA extracted from carp gill tissue was specifically enriched with CyHV-3 sequences through hybridization to a set of nearly 2 million overlapping probes designed to cover the entire length of the genome, using KHV-J sequence (GenBank accession number AP008984) as reference. Even though the rate of enrichment was directly correlated to the initial viral load, results revealed that full genomes could be recovered from gill samples containing as little as 5,000 CyHV-3 copies, with a high depth (>100x) almost all along the genome. Full genome sequences of 18 CyHV-3 specimens or isolates from all over the world as well as 9 nearly-full or partial genomes of atypical specimens were further obtained. These latter included specimens that could not be detected by the OIE-recommended primers and/or that did not elicit the clinical signs classically associated with KHVD. First analyses highlighted a high proportion of intra-specimen sequence heterogeneity, suggesting the presence of mixed infections in many specimens. Comparison of typical specimens showed that genetic diversity at the genome scale was very low (> 99.98% of sequence identity) and confirmed the existence of only two lineages, *i.e.* one Asian and one European. However, some atypical samples exhibited a marked divergence (~3%), which translated into significant alterations of many predicted open reading frames. Analyses are still ongoing, and results will undoubtedly help shed new light on the evolution patterns of this deadly virus, and contribute to the establishment of suitable measures to reduce its incidence (*e.g.* vaccination, eradication program and trade of SPF material).

IDENTIFICATION OF PUTATIVE MICRO-RNAS IN CYHV-3 GENOME AND ANALYSIS OF THEIR EXPRESSION ALONG A LYTIC CYCLE

S. HAMMOUMI[1], C. FOURRE[2], T. VALLAEYS[3], O. RUE[4], C. GASPIN[4], N. KECK[2], J.C. AVARRE[1]*

[1]*Institut de Recherche pour le Développement, Montpellier, France*
[2]*Laboratoire Départemental Vétérinaire de l'Hérault, Montpellier, France*
[3]*Université de Montpellier, France*
[4]*Plate-forme bioinformatique Genotoul-INRA, Castanet-Tolosan, France*

Cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus (KHV) is an emerging virus that causes mass mortality in koi and common carp, *Cyprinus carpio* L. Its 295-kbp genome encodes 156 predicted open reading frames (ORFs), most of them being putative membrane proteins or hypothetical proteins with unknown function (Aoki *et al*., 2007). Gene expression in most herpesviruses involves a complex pattern of transcriptional regulation, and microRNAs (miRNAs) have been shown to play an important role in this regulation. These are short RNAs (around 23 nt long) that regulate gene expression through translational inhibition and mRNA degradation. So far, more than 140 miRNAs have been discovered in herpesviruses. Though the function of the majority of them is still unclear, some have been shown to regulate key steps in herpesvirus lytic and latent cycles (Boss *et al*., 2009).
Applying RNA-seq to short-length RNAs isolated during CyHV-3 infection of carp cells, a set of 64 potential microRNAs was identified. Prediction was based on the length of the corresponding locus in the viral genome, the ability to form a hairpin-like secondary structure and the expression pattern. These predicted miRNAs are spread all along CyHV-3 genome, and almost all of them are located inside ORFs. The matching ORFs correspond to either immediate early or early genes (Ilouze *et al*., 2012). All of these putative miRNAs apparently have a unique sequence in CyHV-3 genome, and some of them seem to be conserved in closely-related herpesviruses such as CyHV-1, CyHV-2 or Anguillid HV-1. Count of the number of occurrences of these 64 loci during the first 10 days of a lytic cycle (2h, 4h, 1 day, 2 days, 3 days, 6 days and 10 days pi) indicated a significant increase after 6-10 days pi for nearly 19 of them. Among these latter, 5 showed a particularly high expression level. None of these 19 candidates could be structurally annotated. Although the genome of *Cyprinus carpio* is not fully sequenced, some of the predicted miRNAs showed a high level of similarity with carp sequences, and a few of them matched with regulatory proteins.

ATLANTIC SALMON, *SALMO SALAR* L., POST-SMOLTS ARE MORE SUSCEPTIBLE TO SALMONID ALPHAVIRUS INFECTION DURING THE INITIAL PHASE AFTER SEA WATER TRANSFER

J. JARUNGSRIAPISIT[1]*, L. MOORE[1], G.L. TARANGER[1], T.O. NILSEN[2], S.O. STEFANSSON[3], P.G. FJELLDAL[1], H.C. MORTON[1], Ø. EVENSEN[4], S. PATEL[1]**

[1]*Institute of Marine Research, Bergen, Norway*
[2]*Uni Research AS, Bergen, Norway*
[3]*University of Bergen, Bergen, Norway;* [4]*Norwegian School of Veterinary Science, Oslo, Norway*
**presenting author, ** corresponding author*

Pancreas disease (PD), caused by Salmonid alphavirus (SAV), results in major financial losses in Atlantic salmon aquaculture. Bath and cohabitation models provide a natural route of infection when studying immune responses and pathogenesis. In our study, Atlantic salmon post-smolts from the same production batch were bath-immersed or intramuscularly injected (i.m.) with SAV subtype 3 (SAV3) either two weeks after (phase-A) or nine weeks after (phase-B) seawater transfer. SAV was detected in blood, heart, and tank-water from all infected groups by real-time PCR. Virus shedding was detected in water collected from tanks containing i.m. infected fish 4 to 28 days post-infection (dpi), peaking at 7 dpi. Shedding from phase-B post-smolts was markedly lower than that from phase-A fish. Necrosis of exocrine cells and cardiomyocytic necrosis was similar in phase-A fish, although there was a time-lag between the i.m. infected fish and the bath-challenged fish. The pathological changes in heart and pancreas from SAV-positive fish in phase-B were less severe compared to post-smolts in phase-A. The prevalence of SAV-positive fish in phase-A and -B bath-challenged groups was 100% and 29%, respectively, whereas it was 96% in i.m. infected groups. The i.m. injection model showed differences in viral loads and pathological lesions between phase-A and -B fish, suggesting differences in host immune responses between these fish, although the same conclusion could not be drawn from the prevalence result. This inconsistency may be due to the artificial route of infection. Gill ATPase activity was significantly lower in both bath and i.m. infected phase-A post-smolts compared to control fish at 21 and 28 dpi, indicating that osmoregulation may also be affected by SAV3 infection. Nevertheless, the levels were still within what is considered normal for post-smolts in seawater. In conclusion, phase-B fish coped with the SAV3 infection better than phase-A fish and this may result in part from their longer exposure to seawater. Using the bath challenge model described in this study, SAV3 was successfully transmitted and caused PD lesions to Atlantic salmon post-smolts. Thus it offers an alternative SAV infection model when a defined viral exposure time and natural route of infection are required.

SUSCEPTIBILITY OF ISOGENEIC GINBUNA *CARASSIUS LANGSDORFII* TO GOLDFISH HEMATOPOIETIC NECROSIS VIRUS (CYHV-2) AS A MODEL SPECIES

A. NANJO[1]*, T. SHIBATA[1], M. SAITO[1], K. YOSHII[1], M. TANAKA[2], T. NAKANISHI[3], T. SAKAMOTO[1], G. KATO[1], M. SANO[1]

[1]*Tokyo University of Marine Science and Technology, Tokyo, Japan*
[2]*College of Bioresource Sciences, Nihon University, Kanagawa, Japan*
[3]*Saitama Prefectural Agriculture and Forestry Research Center, Saitama, Japan*

Herpesviral hematopoietic necrosis (HVHN) caused by goldfish hematopoietic necrosis virus (GFHNV=CyHV-2) gives sever damage to aquaculture of goldfish *Carassius auratus* and gibelio carp *Carassius gibelio* with high mortality. It has been reported that high water temperature treatment can reduce the mortality rate of infected fish and elicit immunity against the virus. Mechanism of the resistance, however, is not well understood at this time. Since clonal ginbuna *Carassius langsdorfii* closely related to goldfish and gibelio carp has been used as a model species for immunological research, we studied susceptibility of ginbuna to GFHNV and effect of high temperature treatment on the mortality of infected ginbuna.

We conducted sensitivity test at 25°C using a clonal ginbuna strain (S3N) and goldfish (Ryukin variety). Fish were inoculated intraperitoneally with GFHNV Sat-1 isolate at a dose of $10^{2.5}$ $TCID_{50}$ per fish. Ginbuna revealed 100% mortality, demonstrating high susceptibility to the virus as well as goldfish. The moribund ginbuna showed diffused and severe necrosis in the hematopoietic tissues. Then we confirmed effect of high temperature treatment on the mortality after viral challenge in clonal ginbuna strain S3N. Ginbuna and goldfish were inoculated with the virus at a dose of 0.3 $TCID_{50}$ per fish and reared at 25°C for 18 h. After high temperature treatment at 34°C for 6 days, fish were reared at 25°C for 2 weeks and then challenged again with the virus as above. All fish died after the first virus inoculation without high temperature treatment, and uninfected-fish treated with high temperature also died after the second challenge. In contrast high temperature-treated infected-fish had no mortality after viral challenge at both virus inoculations.

These data suggest that clonal ginbuna (S3N) is susceptible to GFHNV and acquired resistance to the virus during high temperature treatment. The clonal ginbuna strains can be useful for immunological studies on HVHN as a model.

GOLDFISH ORGAN EXTRACTS CAN ENHANCE PROPAGATION OF GOLDFISH HEMATOPOIETIC NECROSIS VIRUS IN CELL CULTURE

T. SHIBATA*, A. NANJO, M. SAITO, K. YOSHII, T. SAKAMOTO, G. KATO, M. SANO

Faculty of Marine science, Tokyo University of Marine Science and Technology, Tokyo, Japan

Herpesviral hematopoietic necrosis due to goldfish hematopoietic necrosis virus (GFHNV=CyHV-2) causes severe damage to aquaculture of goldfish *Carassius auratus* in Japan and Prussian carp *Carassius gibelio* in China. Several reports mentioned the difficulty in culturing the virus, resulting in total loss of the infectivity within several passages. This difficulty has blocked study on characterization of the virus. In previous study, we reported that supplementation of extract of the healthy goldfish body kidney to medium at a concentration of 0.2% (w/v) can enhance virus propagation in cultured cells. Thus, this protocol makes virus subculture stable and yield of virus solution with high infectious titer. In this study, we investigated effective concentrations of body kidney extract in medium and also effect of supplementation of the extract prepared from other organs of goldfish on virus propagation.

The body kidney and other organs (head kidneys, spleen, hepatopancreas, heart, muscle and serum) of healthy goldfish were homogenized with tenfold volume of MEM-5 followed by centrifugation and filtration with 0.1 μm filter unit. Two kind of virus culture of GFHNV SaT-1 isolate were used: passage 4 (P-4; as a representation of low passage virus) and clone 1 passage 2 (C1-P2) which was prepared from passage 23 (continuously cultured with the extract). The body kidney extract or other organ extract was added to culture medium in 96well plate seeded with a goldfish fin cell line (RyuF-2) at several final concentrations. After inoculation of the tenfold serial dilutions of the virus, the plates were incubated at 25˚C and virus titer was periodically calculated.

The higher concentration of body kidney extract in medium made more rapid development of cytopathic effect of the virus. Final virus titers of C1-P2 with and without supplementation of body kidney extract were the same, while that of P-4 with the extract was over 10 times higher than that without the extract. Supplementation of extract from the other organs made increase of the final virus titer as similar level of that obtained under presence of the body kidney extract.

MACROPHAGES AS MEDIATORS OF (TRAINED) IMMUNITY

G. WIEGERTJES*, I. FINK, J. PETIT

Wageningen University, Wageningen, The Netherlands

Vertebrate macrophages express a range of activation states, with the extremes also termed classical (M1) and alternatively activated (M2) macrophages. The M1-M2 dichotomy could be an intrinsic property of macrophages which arose early in evolution, prior to the development of adaptive immunity. There also is an increasing body of evidence suggesting that innate immune cells such as macrophages possess a form of memory, shown as a heightened response to a secondary infection with the same or a different micro-organism, a phenomenon referred to as trained immunity. For example, human macrophages can be efficiently trained with stimuli such as purified β-glucans. We investigate the evolutionary conservation of M1 and M2 macrophage activation states as well as the phenomenon of trained immunity.

We hypothesized that carp macrophages show activation profiles and markers analogous to those typical of activated human macrophages. Indeed, carp macrophages can polarize into activation states typical of classical (M1) and alternative (M2) extremes. In vitro, cytokine-independent activation with microbial products leads to activated macrophages which display classical M1-type states as shown by high levels of nitric oxide. In contrast, activation with exogenous cAMP drives macrophages to alternative M2-type states as shown by high levels of arginase. Furthermore, cytokine-dependent activation of macrophages with interferon-γ amplifies the classical activation state, whereas activation with interleukin-4/13 leads to high levels of arginase, confirming the M1-M2 dichotomy for carp macrophages. RNA sequencing identified a number of markers for activated, polarized macrophages, several of which are conserved from carp to humans. In addition, we hypothesized that carp macrophages, trained with β-glucans, show activation profiles analogous to those typical of trained human macrophages. To this aim, we measured nitric oxide after in vitro re-stimulation of macrophages with β-glucans and confirm our hypothesis. We expect that both, macrophage activation and trained immunity are mechanisms central to the innate immune system of fish and allow for regulation of immunity via the addition of stimulants to fish feed.

WHAT HAPPENS TO THE INTESTINAL MICROBIOTA OF THE COMMON CARP (*CYPRINUS CARPIO*) WHEN YOU COMBINE ORAL APPLICATION AND INJECTION OF β-GLUCAN?

S. J. HARRIS[1, 2]*, M. ADAMEK[2], N. KAREEM[1, 3], S.L. TAYLOR[1], D. STEINHAGEN[2], D. HOOLE[1]

[1]*Keele University, Keele, UK*
[2]*University of Veterinary Medicine, Hanover, Germany*
[3]*University of Sulaimani, Sulaimaniyah, Kurdistan Region*

The symbiotic relationship between the common carp (*Cyprinus carpio*) and its intestinal microbiota is involved in digestion, homeostasis and preventing infections. There is considerable interest in the modulation of this bacterial community as a means of increasing growth and improving the overall health of fish populations which also reduces the liberal use of antibiotics. Here we show using PCR-DGGE that feeding with a β-1,3/1,6-glucan product (MacroGard®) at a commercially used concentration (0.1% w/v) initially alters the microbiome within the carp gut but does not produce a sustainable change in diversity. This is perhaps not surprising as MacroGard® is marketed as an immunomodulator of the host and a lack of sustainable change in bacterial diversity in healthy fish does not equate to less protection within the gut.
Activation of the immune response does, however, cause a significant change to the intestinal microbiome. Intraperitoneal injection of common carp (*Cyprinus carpio*) with MacroGard® ($2mg/kg^{-1}$ and $5mg/kg^{-1}$) induces a systemic immune response within the intestine capable of significantly reducing expression of the 16S rDNA housekeeping gene, a marker of bacterial activity, by 95% after 24 hours. Although the mechanism behind this reduction is still not known, MacroGard® injection causes significant changes in the gene expression of numerous bactericidal innate immune proteins including CRP, iNOS, C3 and the antimicrobial peptides LEAP2, HAMP1 and ApoA1.
Previous research has thus been studying the effect of MacroGard® on healthy, stable microbial populations. In order to study what happens to a disrupted population, which may occur during an infection or after vaccination, trials are currently being performed to elucidate the effect of oral application of MacroGard® at 0.1% w/v on recolonisation of the gut after a systemic immune response induced by intraperitoneal injection with MacroGard® or heat inactivated bacteria (*Aeromonas salmonicida* subsp. *salmonicida*).
This research may lead to changes in timing vaccines with specific feeding regimes as a means of modulating intestinal microbiota populations in a more effective manner.

THE EFFECT OF IMMUNOMODULATION ON LARVAL SURVIVAL AND IMMUNITY IN TURBOT (*SCOPHTHALMUS MAXIMUS*)

J.J. MIEST[1]*, C. ARNDT[1], M. ADAMEK[2], D. STEINHAGEN[2], T. REUSCH[1]

[1]*GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany*.
[2]*University of Veterinary Medicine, Hanover, Germany*

Immunomodulation has been shown to increase survival and immunity in juvenile and adult fish. Hence the integration of substances such as β-glucan into the feed is already common in order to reduce stress and mortalities in fish farms. However, little is known about the effects immunomodulating substances can have on the most crucial and vulnerable life stage: the larvae. In nature larval survival of some fish species can be as low as 1 %. One aim of aquaculture research is therefore to enhance this survival in order to increase production. In this presentation we present two independent studies: 1) administration of a β-glucan containing feed additive and 2) administration of a nucleotide containing feed additive to turbot larvae (*Scophthalmus maximus*) via life feed (*Brachionus* sp., Rotifers). Daily mortality and growth parameters were monitored and larvae were sampled at 11/12 dph and 24/25 dph. Daily mortality was significantly heightened by β-glucan and nucleotides. Nucleotides increased the dry weight of larvae whilst β-glucan had no effect on growth. In addition we analysed the influence of the feed additive on the expression of genes involved in immunity and metabolism as well as trypsin activity. Our results show that tryptic activity was significantly increased after 11 dph in β-glucan treated larvae and at 25 dph in larvae fed with nucleotides. Effects of the two feed supplements on gene expression differed depending both on timing and supplement. Whilst β-glucan showed immunomodulating effects on both sampling points no such effects were observed after nucleotide administration. Thus our results show the power of larval feed supplementation.

THE EFFECT OF A PROBIOTIC DELIVERED BY THREE DIFFERENT ROUTES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AGAINST THREE DIFFERENT PATHOGENS

S. LA PATRA*, W. SHEWMAKER, R. BURKHART, A. MORTON

Clear Springs Foods, Inc., Buhl, ID USA

To determine the potential for protection against infectious hematopoietic necrosis virus (IHNV), *Flavobacterium psychrophilum* and *Yersinia ruckeri* induced mortality, a study was conducted where a probiotic, *Enterobacter* sp. strain C6-6, was delivered to rainbow trout, *Oncorhynchus mykiss*, via either intranasal (IN), intramuscular (IM) or intraperitoneal injection (IP). Groups of rainbow trout (mean weight, 3 g) were either injected with PBS or a standardized concentration of C6-6 and subsequently challenged with three different pathogens at 9 and 30 days post-injection (PI). At 9 days the relative survival (RS) ranged from 0 to 90%. The fish injected with the probiotic, regardless of the delivery route, actually appeared more susceptible to IHNV then the PBS injected controls. However, RS values after being challenged with *F. psychrophilum* were 90 and 84% after IP and IM injection of C6-6, respectively. A similar trend was observed after the treated fish were challenged with *Y. ruckeri* and RS values of 90 and 79% were observed after IP and IM injection. No significant protection against any of the pathogens was observed after IN delivery of the probiotic at 9 days PI. At 30 days PI the RS ranged from 0 to 83%. The RS values after being challenged with IHNV were 52 and 62% after IP and IM injection of C6-6, respectively. The RS values after being challenged with *F. psychrophilum* were 83, 78 and 52% after IP, IM and IN injection. However, less protection was observed after the treated fish were challenged with *Y. ruckeri* and had RS values of 5 and 52% after IP and IM injection. Again, IN delivery of the probiotic provided no significant protection after challenge with IHNV and *Y. ruckeri* at 30 days PI. Taken together, the results from this study indicate that protection against IHNV, *F. psychrophilum* and *Y. ruckeri* after either IM or IP injection of this naturally occurring bacterium, is at least in part dependent on some enhanced immune function(s) in the treated fish. *Enterobacter* sp. strain C6-6 may be useful as a potential alternate strategy for reducing the impacts from bacteria and possibly viral infections through non-specific immune-enhancement during times of increased fish stress or as a possible adjuvant.

EFFECTS OF PREBIOTIC AND PROBIOTIC SUPPLEMENTATION ON GROWTH PERFORMANCE AND GUT MORPHOLOGY OF EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*) JUVENILES

F. RIVERO*, M.J. CABALLERO, S. TORRECILLAS, M.S. IZQUIERDO, D. MONTERO

Grupo de Investigación en Acuicultura (GIA), Universidad de Las Palmas de Gran Canaria, Parque Científico-Tecnologico Marin, Muelle de Taliarte s/n. Telde, Las Palmas, Canary Islands, Spain

The use of dietary pre- and pro-biotics (functional diets) for marine fish has been proved to promote fish performance in relation to growth and feed efficiency, and also to reinforce the intestinal barrier in terms of integrity, functionality and increased mucus production together with stimulatory effects of intestine immune system. The substitution of fish oil by vegetable oils has been shown to reduce growth and to affect both immune system and gut integrity in marine fish. Thus the objective of this study was to evaluate the use of functional diets to improve the gut integrity of European seabass juveniles. One thousand and eight hundred European seabass juveniles (19.7±0.09g mean weight) were randomly allocated in 18 tanks of 500 l and fed six iso-energetic and isolipidic diets with different levels of prebiotics and probiotics for 123 days. Diets 1 and 2 contained high or low levels of prebiotics, respectively. Diet 3 contained probiotics, whereas Diet 4 and 5 contained probiotics plus high or low prebiotic inclusion, respectively. Diet 6 was formulated with no addition of pre- or probiotics.

All groups were fed 3-times a day, 6 days a week, by hand until apparent satiation. At days 57 (S1) and day 87 (S2), 4 animals per tank (12 per diet) were sampled and posterior intestine (from diffuse sphincter to rectal sphincter) was removed for histological (H&E and alcian-blue stains) and immunohistochemistry (TNF and iNOS) analysis. Growth was also monitored at day 123 (S3).

At the end of experiment fish from D2 and D5 had significantly ($p<0.05$) higher weight and length than those from D6. Besides, fish fed D5 showed the highest ($p<0.05$) number of goblet cells in posterior intestine. INOS and TNF immunoreactivity was found in intestinal cells located at basal levels of intestinal villi, as well as in cellular infiltrates located in the submucosa layer, whereas mucosa cells showed only iNOS immunoreactivity.

STUDY ON USING OF *CHLORELLA VULGARIS* AS NATURAL IMMUNOSTIMULATOR IN ORDER TO INCREMENT OF IMMUNITY SYSTEM AGAINST VIRAL NERVOUS NECROSIS (VNN) AS NEW EMERGING DISEASE IN CASPIAN BROWN TROUT (*SALMO TRUTTA CASPIUS*)

A. SABERI[1], M.E.J. ZORRIEHZAHRA[2]*, H. EMADI[1], SH. KAKOLAKI[2], S.M.R FATEMI[1]

[1]*Islamic Azad University, Research & Sciences Branch, Tehran, I.R. Iran*
[2]*Iranian Fisheries Research Organization (IFRO), Tehran, I.R. Iran*

Climatic changes influence the emergence and reemergence of infectious diseases, in addition to multiple human, biological, and ecological determinants. Climatologists have identified upward trends in global temperatures and now estimate an unprecedented rise of 2.0 degrees C by the year 2100. Of major concern is that these changes such as ocean warming and acidification can affect the introduction and dissemination of many serious infectious diseases to human, animal, plants and marine fishes. Recently some viral emerging diseases were occurred in aquaculture and mariculture in the region. VNN is a new emerging disease that has been reported in most parts of the world .VNN is caused by Nodaviridae family (Betanodavirus) and attacks the nervous system of the fish. At present, there is neither a treatment nor a vaccine available to prevent VNN in fish so in current study we examined a kind of marine algae (*Chlorella vulgaris*) as natural immunostimulator in order to increment of the immune system of Caspian brown trout (*Salmo trutta caspius*). To survey the effect of *Chlorella vulgaris* on *Salmo trutta caspius*, Immune and blood parameters of fish and its sensitivity to VNN were examined. Four treatments including control, T1, T2 and T3, with three replications were designed and feed during sixty days with different doses of chlorella, 0, 1×10^8, 2×10^7 and 3×10^6 chlorella/Kg food respectively. Blood samples were collected at the end of experiment and Immune and blood parameters were measured. In addition, the virus supernatant was prepared using infected *Liza aurata* and was used for intraperitoneal prechallenge with using of guppy and Brown trout. Although disease symptoms and mortalities were observed in guppy, no symptoms and mortalities were observed in Caspian Sea brown trout. Also, final challenge was done intraperitoneally in the four treatments after sixty days of feeding. Blood samples were collected 14 days after the final challenge to measure immune and blood parameters in the control group and the feed treatments by chlorella. Results of prechallenge showed the supernatant has enough virulence and final results determined the effect of chlorella on immune system and blood parameters were improved in Caspian Sea brown trout and revealed that this species of fish is resistant to VNN virus in the Caspian Sea.

DIETARY SUPPLEMENTATION WITH THE Δ5 DESATURASE MUTANT OF *LOBOSPHAERA INCISA* (MICROALGAE) IN GUPPIES: EFFECT ON IMMUNE FUNCTION AND RESISTANCE TO INFECTION WITH *TETRAHYMENA* SP.

I. PORTUGAL*, I. KHOZIN-GOLDBERG, S. BOUSSIBA, D. ZILBERG

The Jacob Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Midreshet Ben Gurion, Israel

Losses associated with diseases and stress pose significant burden in the aquaculture industry and use of appropriate immune-modulatory dietary supplements can serve as a partial solution. The arachidonic acid (20:4 *n*-6) deficient Δ5 desaturase mutant of *Lobosphaera incisa* (code name P127) is the richest green source of an omega-6 LC-PUFA, dihomo-γ-linolenic acid (DGLA). Being the precursor for anti-inflammatory eicosanoids, DGLA is assumed to induce an anti-inflammatory effect. Its benefit has been demonstrated in several studies with mammals, but had never previously been tested in fish.

A study was conducted to assess the effects of the DGLA-rich microalgae P127 in guppies (*Poecilia reticulate*). Fish were fed a diet supplemented with different levels of P127, containing 5%, 10% and 20% of broken dry algae, providing final DGLA concentrations of 20, 40 and 80 µg per gram food, respectively. The non-supplemented commercial food served as control. Fish were fed with the different diets for a period of 6 weeks at 2% of their body weight per day. Mortality was monitored throughout. After 3 weeks of feeding, fish were challenged with *Tetrahymena* sp., a parasite that is considered to be a significant disease problem in guppies. Fish were sampled for immune analyses prior to the challenge and for fatty acid analyses prior to the challenge and 1 week after challenge.

Mortality during the 3 weeks prior challenge was 14.5% and 25.5% in the 20% supplemented and control groups, respectively. Similarly, cumulative mortality following challenge with *Tetrahymena* sp.in the 20% supplemented group was significantly lower than in the control (32.8% and 52.1%, respectively). An analysis of innate immune function and fatty acids is underway.

THE ECONOMIC COST OF PARASITIC DISEASE IN AQUACULTURE

G. PALADINI[1]*, A.P. SHINN[2], J. PRATOOMYOT[3], J.E. BRON[1], E.E. BROOKER[1], A.J. BROOKER[1]

[1]*Institute of Aquaculture, University of Stirling, Stirling, UK*
[2]*Fish Vet Group Asia Limited, Bangkok 10210, Thailand*
[3]*Institute of Marine Science, Burapha University, Chonburi, Thailand*

Obligate and opportunistic parasites play a critical role in determining the productivity, sustainability and economic viability of the global finfish and shellfish aquaculture industry. Without stringent and appropriate control measures, the impact of these pathogens can often be significant. Estimation of the impact of parasites in aquaculture represents a considerable challenge as costs may be affected by a diverse assortment of environmental and management factors and can range from direct losses in production, to the more indirect costs of longer term control and management of infection. The purpose of the current study was to estimate the potential global economic costs attributable to a range of key parasite pathogens using a number of specific events for the purposes of illustration and estimation of costs. The study examines parasite-associated events recorded for the world's major marine, brackish and freshwater aquaculture production industries in order to provide a baseline resource for risk assessment and the development of more robust biosecurity practices. Such developments can, in turn, help to mitigate against and / or minimise the potential impacts of parasite-mediated disease in aquaculture.

MONITORING PARASITE INCIDENCE IN GILTHEAD SEA BREAM HELD IN EXPERIMENTAL AND PRODUCTION CAGES: LONG-TERM EFFECT OF A SUPPLEMENTED DIET ON FISH HEALTH

O. PALENZUELA[1]*, C.L. BROWDY[2], Y. PETROPOULOS[3], N. KATRIBOUZAS[3], R. DEL POZO[1], A. SITJÀ-BOBADILLA[1]

[1]*Institute of Aquaculture "Torre la Sal" (IATS-CSIC), Castellón, Spain*
[2]*Novus International Ltd., Saint Charles, MO, USA*
[3]*Andromeda Group, Athens, Greece*

Gilthead sea bream (GSB) production in net cages is hampered by the incidence of parasitic diseases. The monogenean blood-sucking *Sparicotyle chrisophrii* and the emaciating intestinal myxozoan *Enteromyxum leei*, are the most widely reported pathogenic parasites in these conditions, and tools for their prevention and control in production conditions are rather limited. Lack of effective and licensed treatments and pressure for a smaller environmental footprint of the activity has stimulated the development of bioactive additives and supplemented feeds as a possible strategy to mitigate health problems in cage-cultured fish. Some of these products have clearly demonstrated biocidal and/or immunomodulating activity at laboratory scale with certain host-pathogen models. However, there is a significant lack of corroborating data from commercial scale cage production conditions, wherein fish must cope with multiple, sustained biotic and abiotic stressors. Since different parasites exploit different host niches using a wide repertoir of pathogenic mechanisms, it is important to evaluate the possible benefits of such strategies under real world conditions.

In this study, groups of GSB fed a control and an experimental diet with commercial prebiotics and active ingredients (contained in Previda® and NextEnhance® supplements) were reared in triplicate experimental cages in Western Greece. Biometrical, haematological and parasitological data (focused on prevalence and abundance of gill monogeneans *Sparicotyle chrisophrii* and *Furnestinia echeneis*) was collected monthly (9 samplings along 14 months), with additional intermediate and final samplings analysing intestinal and kidney endoparasites. In addition, subsets from both groups were transferred to full-size production cages, and reared to commercial size (roughly 16 months). At harvest, these fish were also sampled for parasites, including a quantification of *Enteromyxum leei* load on the intestines using qPCR.

Analyses of biometrical, haematological and parasitological data from both groups are presented and the differences between both fish groups are discussed. While certain differential effects can be observed with the feeds tested, the study highlights the difficulties to translate sensible nutritional prophylaxis strategies into immediate solutions for complex, multifactorial processes such as different host-parasite relationships.

This work was partially supported by research grants PROMETEOII/2014/085 and ISIC 2012/003 (Generalitat Valenciana), AQUAEXCEL (EU FP7-262336), and CSIC Internal Research Programs (Intramural project 201330E025)

HISTOLOGICAL EFFECTS AND IMPACT OF JUVENILE SPECIMENS OF *SPARICOTYLE CHRYSOPHRII* AND *ZEUXAPTA SERIOLAE* ON CULTURED GILTHEAD SEABREAM AND GREATER AMBERJACK FROM THE MEDITERRANEAN

A. REPULLÉS-ALBELDA[1]*, F. PADRÓS[2], J.A. RAGA[1], F.E. MONTERO[1]

[1]*Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain*
[2]*Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Barcelona, Spain*

Sparicotyle chrysophrii infecting gilthead seabream (*Sparus aurata*) and *Zeuxapta seriolae* parasiting greater amberjack (*Seriola dumerili*) in the Mediterranean are two species of polyopisthocotylean monogeneans which have been associated to massive infections in cultures and high economical losses. Pathologies caused by adult specimens of *S. chrysophrii* and *Z. seriolae* have been described but histological damages caused by juveniles remain unknown. The aim of this study is to describe pathological effects of juveniles of these two monogenean species in order to provide useful insight to manage infections in cultures. To achieve this objective, 68 gilthead seabreams and 22 greater amberjacks were infected and killed periodically in order to study their gills. The arches of the left side were examined in fresh while those of the right side were fixed and preserved in formaline 10% for subsequent histological analyses. Early juveniles of both species have a simple haptor bearing two pair of hooks, which perforate the gill tissue surface. Despite morphological similarities, effects on gill tissue were different: whereas no specific reaction was observed for *S. chrysophrii,* an inflammatory response was detected around the insertion of *Z. seriolae* hooks. Haptor developed adding clamps. Juveniles, combining hooks and clamps, introduced their hooks between two lamellae and grabbed with their clamps the following ones. The previous inflammatory response to *Z. seriolae* seemed to weaken and individuals of both species attached their haptor with no apparent tissular reaction of their hosts until hooks felt down. *S. chrysophrii* adults developed up to 70 pairs of clamps arranged symmetrically while *Z. seriolae* adults had an asymmetric haptor with 40/30 clamps in the long and short side respectively. Effects were then analysed in terms of affected gill area although according to the different clamp sizes, up to three gill lamellae could be grasped causing lamellar fusion and hyperplasia. Finally, a remarkable finding was that number of parasites decreased with the ongoing infection. Therefore, it is quite likely that controlling the higher abundance and potential effects of juveniles, rather than adults, represents the real challenge.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

WITHDRAW O-016

OCCURRENCE OF THE RENAL SPHAEROSPOROSIS CAUSED BY *SPHAEROSPORA EPINEPHELI* IN CAGE-CULTURED ORANGE-SPOTTED GROUPER *EPINEPHELUS COIOIDES* IN SOUTH CHINA SEA

L.W. XU[1], X.H. LIU[2], J.Y. ZHANG[2]*, J. FENG[1]

[1]*South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, PR China*
[2]*Fish Diseases Laboratory, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China*

In November of 2011, the renal sphaerosporosis was found in cage-cultured orange-spotted grouper *Epinephelus coioides* in a fish farm in Shuidong town, Guangdong province, China. The infected fish exhibited emaciation, anaemia and anorexia, slightly splenomegaly and renomegaly, occasionally with skin ulcer. Mixed infection with *Pseudorhabdosynochus epiepheli* in the gill was generally found, but with low infection intensity. The disease occurred when water temperature ranged from 21º to 27º C and the cumulative mortality ranged from 50% to 80% within 2 weeks. The renal tubules were the only target tissue of the parasite and completely occluded by sporogonic pseudoplasmodia of various degrees of maturity. Many sporogonic stages were attached to the brush border of the epithelium of the renal tubles and mature spores predominately located in the lumen of the tubules. No pseudoplasmodia and mature spores were found in the glomeruli capillary and Bowman's space of the infected kidney. No bacteria were isolated from the diseased fish. Combined with morphological characteristics, histopathological and microbiological analysis, molecular data and host, infection site and geographical distribution, this myxosporean can be identified to be *Sphaerospora epinepheli* and was suspected to be the etiologic agent of this disease for its' high infection prevalence and intensity. Sequence analysis showed that an insertion of "GGTGG" in the V4 E23_15 region of SSU rRNA gene sequence of *S. epinepheli* isolates from *E. malabaricus*, which can differentiate it from isolates from *E. coioides*. This is a new locality record of *S. epinepheli* and first report of *Sphaerospora* species in South China Sea. Additionally, the validity of *Sphaerospora koreana* (Cho & Kim, 2001) n. comb was discussed.

RNA-SEQ ANALYSIS OF INCIPIENT ENTEROMYXOSIS IN TURBOT (*SCOPHTHALMUS MAXIMUS*)

P. RONZA[1]*, D. ROBLEDO[2], A.P. LOSADA[1], B.G. PARDO[1], R. BERMÚDEZ[1], P. MARTÍNEZ[1], M.I. QUIROGA[1]

[1]Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain [2]Facultad de Biología (CIBUS), Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Enteromyxum scophthalmi, an intestinal myxozoan parasite, is the causative agent of a threatening disease for turbot (*Scophthalmus maximus*, L.) aquaculture. The colonization of the digestive tract by the parasitic forms leads to a cachectic syndrome associated to high morbidity and mortality rates. The pathogenetic mechanisms acting in the early stages of infection are still not fully understood. This myxosporidiosis presents a long pre-patent period; also, the first detectable histopathological changes are subtle. Further information would help to implement efficient preventive and therapeutic measures. In order to gain insights into incipient enteromyxosis, we performed a transcriptome analysis of kidney, spleen and pyloric caeca from experimentally-infected and control turbot using RNA-Seq. A histological evaluation was carried out to select the specimens to be analyzed, and turbot presenting very early signs of infection were included. As well, the presence of *E. scophthalmi* was confirmed by immunohistochemistry. RNA-Seq analysis revealed, as expected, less intense transcriptomic changes compared with those found in late stages of the disease. Up-regulated expression of several genes related to interferons was found in the three organs, demonstrating that interferon-mediated innate immune response may be involved in this phase of the disease. Interestingly, an opposite expression pattern had been found in severe infected turbot. Also, the three organs showed some evidence of activation of acquired immunity, with the up-regulation of genes related with T- and B-cells. In pyloric caeca, the antigen-presenting cell marker CD209 showed an increased expression, a common finding with the late infection. Curiously, down-regulation of different genes encoding acute-phase proteins and protease inhibitors was detected, especially in spleen. Moreover, numerous genes related with cell proliferation and differentiation were differentially expressed at intestinal level, showing a possible inhibition of both processes in early infection. On the other hand, genes codifying for proteins of cell junctions and extracellular matrix were up-regulated in this location. The results of this work contribute to a better knowledge of turbot enteromyxosis, helping to elucidate the events that characterize the early stages of infection. As well, the study provides valuable information to identify molecular markers for early detection and control of this important parasitosis.

This work was funded by the Spanish Ministry of Science and Innovation through the project AGL2009-13282-C02-02. P. Ronza holds a grant by the scientific network "INMUNOGENOM", funded by Xunta de Galicia (REDES GI-1251).

DIFFERENTIAL SUSCEPTIBILITY OF YUKON RIVER AND SALISH SEA CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) STOCKS TO *ICHTHYOPHONUS*

D.G. ELLIOTT[1]*, C.L. MCKIBBEN[1], C.M. CONWAY[1], A. MACKENZIE[2], L.M. HART, M.K. PURCELL[1], P.K. HERSHBERGER[2]

[1]*U.S. Geological Survey, Western Fisheries Research Center, Seattle, Washington, USA*
[2]*U.S. Geological Survey, Marrowstone Marine Field Station, Nordland, Washington, USA*

The fish parasite *Ichthyophonus* sp. has been hypothesized as a driver of long-term declines in Chinook salmon (*Oncorhynchus tshawytscha*) in the Yukon River (northwestern North America) by contributing to pre-spawning mortality. Interestingly, ichthyophoniasis has not been a reported problem in Chinook salmon outside the Arctic-Yukon-Kuskokwim region, despite occurring in high prevalence among prey fishes throughout the eastern North Pacific Ocean, including the Salish Sea (Washington State, USA). This study was intended to address this apparent paradox by comparing relative susceptibilities of Yukon River- and Salish Sea-origin Chinook salmon to *Ichthyophonus*. Juvenile Chinook salmon from Yukon River and from Kendall Creek (Salish Sea) stocks were exposed to *Ichthyophonus* in fresh water during two 6-day trials by feeding with minced tissues from infected Pacific herring (*Clupea pallasii*). Control groups were fed with tissues from specific pathogen-free herring. Periodic subsampling for *Ichthyophonus* detection was conducted up to 65 days after initial exposure. Infection prevalence was determined by microscopic examination of heart and liver explant cultures for characteristic schizonts, and disease progression was evaluated by histopathology. A third feeding trial with the two fish stocks was conducted in seawater. *Ichthyophonus*-related mortality was $\leq$ 26% in Yukon River-origin salmon and rarely observed in Kendall Creek-origin salmon. *Ichthyophonus* infection rates were higher in Yukon River fish than in Kendall Creek fish ($P \leq 0.04$) during all three trials. Initial infection rates up to 100% and 73% occurred in the Yukon River and Kendall Creek treatments, respectively. Infection prevalence remained near initial levels in Yukon River fish throughout the post-challenge holding period, but prevalence decreased to 0 – 8% in Kendall Creek fish by the end of each experiment. Culture and histopathology results indicated that *Ichthyophonus* caused persistent and progressive infections in Chinook salmon from the Yukon River but more transient infections in those from Kendall Creek.

ANALYSIS OF THE ENDOCRINE HORMONE SYSTEM IN THE SALMON LOUSE: POSSIBLE TARGETS FOR CHEMICAL INTERVENTION?

L. SANDLUND[1]*, F. NILSEN[1], R. MALE[2], S. DALVIN[1, 3]

[1]*Sea Lice Research Centre, University of Bergen, Bergen, Norway*
[2]*Sea Lice Research Centre, University of Bergen, Bergen, Norway*
[3]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*

The salmon louse *Lepeophtheirus salmonis* (Copepoda, Caligidae) is a major disease in the salmon farming industry in the Northern Hemisphere causing annual losses of hundreds of million US dollars world-wide. Due to emerging resistance problems towards the treatments methods available on the market today it is essential to develop new methods for lice control. In order to facilitate development of chemotherapeutants and gain control of the parasite, knowledge about molecular biological functions of *L. salmonis* is vital. In arthropods, developmental processes such as reproduction, oogenesis and molting are mediated by binding of steroid hormones, ecdysone, to a heterodimer of the nuclear receptors, ecdysone receptor (EcR) and a homolog of the retinoid X receptor, ultraspiracle (USP). We have preformed RNAi-mediated knock down of genes which codes for ecdysteroid biosynthetic enzymes and the nuclear receptor complex. The findings from these studies will be presented and discussed.

CAN EXPRESSION LEVELS OF MUCIN AND CELL-CELL CONTACT GENES BE USED AS AN INDICATOR OF MUCOSAL IMMUNE RESPONSES?

M. ADAMEK[1]*, D. HAZERLI[1], M. MICHALI[1], G. BROGDEN[1], M. MATRAS[2], D. STEINHAGEN[1]

[1]*Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2]*Laboratory of Fish Diseases, National Veterinary Research Institute, Puławy, Poland*

Healthy skin protects fish against invading pathogens and physical stresses from the surrounding environment. The first line of physical protection is mucus - a gel like cover of the epithelium. Transmembrane and gel forming mucins are the most abundant glycoproteins present in mucus. The second line of defence is the skin epithelium which regulates paracellular tightness via the cell to cell contact proteins of tight and adherent junctions and desmosomes. During infection these structures can be challenged, lowering the skin's defence potential.
In order to monitor mRNA expression of common carp mucins, an early version of the common carp genome was used to describe genes encoding for the transmembrane mucins 13 and 18 and the secreted mucins 2-like and 19. Furthermore, fragments of genes encoding for cell contact proteins (tight junction protein 2B, cadherin 1, desmocollin 2, occludin) were amplified based of expressed sequence tags. The expression was measured with RT-qPCR in carp skin, gill and gut during a *Cyprinid herpesvirus 3* (CyHV-3) infection. For mucins, additional feeding trials with different concentrations of MacroGard® (β-1,3/1,6-glucan) and an intraperitoneal injection of poly I:C was performed. Also, an *in vitro* model for carp skin based on fin and scale primary cell cultures was developed and used to confirm *in vivo* findings on both mRNA and protein level. Additionally, an attempt to raise an epithelial cell line from carp skin was made in order to further improve the *in vitro* model.
Results showed that a CyHV-3 infection influences adherent junctions and desmosomes by down-regulating the expression of cadherin 1 and desmocollin 2. Observations on primary cell cultures confirmed these results. A novel epithelial cell line could not be established because fibroblasts overgrew the epithelial cells. Expression of mucins was also down-regulated during CyHV-3 infection and poly I:C injection. Our results indicate that the expression of mucins and cell contact proteins cannot be used as a reliable indicator for mucosal immune responses. However results indicate that CyHV-3 infection remodels and weakens the epithelial barrier, which can foster tissue penetration and fish to fish transmission of the virus as well as susceptibility to secondary infections.

ANTIGEN SAMPLING CELLS IN GILL EPITHELIUM OF RAINBOW TROUT

G. KATO[1]*, T. YAMAGUCHI[2], U. FISCHER[2]

[1]*Tokyo University of Marine Science and Technology, Tokyo, Japan*
[2]*Friedrich-Loeffler-Institut, Greifswald, Germany*

As reported in Japanese flounder and rainbow trout, antigens are taken up by antigen sampling cells in gill epithelium during bath vaccination. However, the phenotype of antigen sampling cells is largely unknown. Microfold (M) cells, antigen sampling cells in mammalian mucosal tissues, are typically recognized by the lectin *Ulex europaeus* agglutinin-1 (UEA1), but not by wheat germ agglutinin (WGA). In this study, a lectin binding assay and flow cytometry of the antigen sampling cells was performed to characterize the phenotype of antigen sampling cells in gill epithelium.
Rainbow trout were bath-vaccinated with *Aeromonas salmonicida* subsp. *salmonicida* (*A.s.s.*) bacterin and their gills were removed and fixed in Davidson's fixative. Fixed tissues were then embedded into paraffin, sectioned and stained with anti-*A.s.s.* antibodies to detect the antigen sampling cells. The sections were additionally stained with UEA1 or WGA on the same slide. Furthermore, gills were sampled from rainbow trout bath-vaccinated with Syto61-stained *A.s.s.* bacterin. Cells were isolated from gill epithelium and stained with UEA1 and monoclonal antibodies against rainbow trout leukocyte subpopulations (anti-CD8α, anti-IgM and anti-thrombocyte). The stained cells were then subjected to flow cytometry.
In the gills of bath-vaccinated fish, uptake of *A.s.s.* bacterin was observed in the epithelium at the base of primary lamellae where the interbranchial lymphoid tissue (ILT) is located, and in secondary lamellae. Most of the antigen sampling cells were also recognized by UEA1 but not by WGA. Flow cytometry analyses revealed that approximately 15 % of the gill epithelial cells were positive for the Syto61-stained bacteria in bath-vaccinated fish. More than 70 % of the $Syto61^+$ cells were also stained by UEA1, whereas hardly any $CD8\alpha^+$, IgM^+ cells or thrombocytes were found among $Syto61^+$ cells. According to forward scatter characteristics, $Syto61^+$ $UEA1^+$ cells were mainly found in a cell fraction which was larger than both lymphocytes and monocyte/macrophages. In conclusion, our data suggest that non-leukocyte $UEA1^+$ antigen sampling cells from the gill epithelium display features of mammalian M cells.

PROTEOME OF GILTHEAD SEABREAM (*SPARUS AURATA*) SKIN MUCUS AFTER PROBIOTIC FEEDING AND CROWDING STRESS

H. CORDERO[1, 2]*, P. MORCILLO[1], A. CUESTA[1], M.F. BRINCHMANN[2], M.A. ESTEBAN[1]

[1]*University of Murcia, Murcia, Spain*
[2]*University of Nordland, Bodø, Norway*

Gilthead seabream (*Sparus aurata*) is a major aquaculture species in the Mediterranean area. High density stocking could cause some emerging diseases triggering important economic losses. Probiotics are a promising solution to prevent diseases through several mechanisms such as improving the immune status and/or mucosal microbiota or competing with pathogens. Probiotic *Shewanella putrefaciens*, also known as Pdp11, was firstly isolated from the healthy skin of gilthead seabream. Our study is focused on the skin mucus proteome after dietary probiotic Pdp11 in fish maintained under normal or crowding cultured conditions. For that, 2-DE followed by LC-MS/MS analysis was done in skin mucus for each experimental group and differentially expressed proteins were identified. Results showed differentially expressed proteins involved in immune processes (such as lysozyme, complement c3, natural killer enhancing factor), metabolism processes (inositol monophosphate and apolipoprotein A1) and cellular structure (profilin and beta actin). To our knowledge this is the first time that the proteome of gilthead seabream mucus is studied after stress conditions and probiotic feeding. Further researches are necessary for unravelling the implications of these proteins in skin mucosal immunity.

H. Cordero wishes to thank MINECO and FEDER for a FPI grant. This work has been supported by projects of MINECO (AGL2011-30381-C03-01) and Fundación Séneca de la Región de Murcia (Grupo de Excelencia 04538/GERM/06).

MULTIPLE IRAK3 VARIANTS ARE ENCODED IN SALMONID FISH: THE FUNCTIONAL COPIES INHIBIT PATHOGEN-DEPENDENT SIGNAL TRANSFER

A. REBL[1]*, M. VERLEIH[1], R.M. BRUNNER[1], H. REBL[2], H.-M. SEYFERT[1], T. GOLDAMMER[1]

[1]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany*
[2]*Department of Cell Biology, Rostock University Medical Center, Rostock, Germany*

The control of cellular immune processes is mandatory to avoid detrimental pathological responses. IRAK3 (Interleukin-1 receptor-associated kinase 3) is known as a negative feedback regulator of the mammalian Toll-like receptor (TLR) signaling cascade dampening the transfer of pathogen-induced inflammatory signals. Since irak3 orthologs have not been characterized in teleost species so far, we searched 332 million RNA-seq reads generated with an Illumina Genome Analyzer exhaustively characterizing the transcriptome of six different tissues from rainbow trout (Oncorhynchus mykiss). The resultant consensus sequence encoding trout Irak3 features 23 single nucleotide variations (SNVs) including 13 non-synonymous mutations. However, these SNVs were present in various combinations on individual irak3 sequencing reads. We therefore cloned Irak3-encoding sequences from the salmonid fishes rainbow trout and maraena whitefish (*Coregonus maraena*) confirming the presence of still 11 SNVs. The comparison of protein lengths indicated that the salmonid Irak3 proteins comprise significantly less amino acid residues than its mammalian counterparts, although typical domains are present in salmonid Irak3 factors.
We expressed the most abundant trout Irak3 variants in human HEK-293 cells, a model cell line to investigate aspects of the TLR signaling cascade. Expression of a GFP-tagged trout Irak3 revealed, expectedly, its cytoplasmic localization in HEK-293 cells. This factor significantly elevated in a dose-dependent manner the basal level of NF-kappaB, a factor crucial for the initiation of inflammatory events. After pathogen stimulation, Irak3 blunted the TLR-dependent activation of NF-kappaB. These reporter gene assays thus indicate the functional conservation of Irak3 factors in mammals and salmonids.

This project is funded by the European Fisheries Fund (EFF) and the Ministry of Agriculture, the Environment and Consumer Protection Mecklenburg-Western Pomerania, Germany (Pilot project VI-560/730-32614).

TYPE I INTERFERONS SHOW STRONG ADJUVANT ACTIVITY IN DNA VACCINATION OF ATLANTIC SALMON AGAINST INFECTIOUS SALMON ANEMIA VIRUS

C.J. CHANG*, B.J. SUN, B. ROBERTSEN

Norwegian College of Fishery Science, University of Tromsø, Tromsø, Norway

There is a need for more efficient vaccines to combat viral diseases of Atlantic salmon and other farmed fish. DNA vaccines are highly effective against salmonid rhabdoviruses, but have shown less effect against other viruses. In the present work we have studied if type I IFNs might be used as adjuvants in fish DNA vaccines. For this purpose we chose a DNA vaccine model based on the hemagglutinin esterase (HE) gene of infectious salmon anaemia virus (ISAV) as antigen. Salmon presmolts were injected with a plasmid encoding HE alone or together with a plasmid encoding Atlantic salmon type I IFN (IFNa, IFNb or IFNc). Sera were harvested after 7-10 weeks for measurements of antibody against ISAV and the fish were challenged with ISAV to measure protective effects of the vaccines. The results showed that all three IFN plasmids delivered together with HE plasmid potently enhanced protection of salmon against ISAV mediated mortality and increased production of IgM antibodies against the virus. In contrast, HE plasmid alone gave low antibody titres and a minor protection against ISAV. This demonstrates that type I IFNs stimulate adaptive immune responses in fish, which may be a benefit also in other fish DNA vaccines. Quantitative RT-PCR analyses suggest that the IFNs cause an increased influx of B-cells and cytotoxic T-cells at the muscle injection site, which may in part explain the adjuvant effect of the IFNs.

SENEGALESE SOLE (*SOLEA SENEGALENSIS*) ISG15: MOLECULAR CHARACTERIZATION AND *IN VIVO* INTERPLAY WITH VIRAL INFECTIONS

D. ALVAREZ-TORRES[1]*, A.M. PODADERA[1], I. BANDIN[2], J. BEJAR[1], E. GARCIA-ROSADO[1], M.C. ALONSO[1]

[1]*Universidad de Málaga, Málaga, Spain*
[2]*Universidad de Santiago de Compostela, Santiago de Compostela, Spain*

The interferon-stimulated gene 15 (*Isg15*) is strongly induced by type I interferon (IFN I), viral infection, and double-stranded RNA (poly I:C) in several fish species, suggesting that Isg15 protein could play a key role in fish innate immunity against viral diseases. Thus, the aim of the present study was to characterize the molecular structure and transcription pattern of the Senegalese sole (*Solea senegalensis*) *Isg15* gene in response to viral infections.
The molecular characterization shows that the Senegalese sole *Isg15* gene codes for a typical Isg15 protein of 165 aa, containing two ubiquitin-like domains and one conserved LRLRGG conjugating motif at the C-terminal end. The untranslated 5´-end region exhibited the structure of an IFN-stimulated gene promoter, with two interferon stimulated response elements (ISRE). Pairwise alignments based on deduced amino acid sequences showed homologous relationships (72.5-74.2%) between the Isg15 of Senegalese sole and other pleuronectiforms.
The *Isg15* transcription has been studied in head kidneys of Senegalese sole inoculated with poly I:C and with different fish viruses: two Viral Haemorrhagic Septicaemia Virus (VHSV) isolates (highly pathogenic and non-pathogenic to sole), and one reassortant Viral Nervous Necrosis Virus (VNNV) isolate, composed of a RGNNV-type RNA1 and a SJNNV-type RNA2 (pathogenic to sole). These challenges showed that poly I:C induces *Isg15* transcription from 3 to 72 h post-injection (p.i.), whereas the induction in response to viral infections started at 24-48 h p.i. The fast induction of *Isg15* indicates the potential implication of this ISG in the antiviral state stablished by the IFN I system. On the other hand, the interaction between each virus and the IFN I system was evaluated in fish inoculated with poly I:C and subsequently (24 h later) challenged with the different viruses. This challenge showed a viral multiplication decrease in poly I:C treated animals compared with untreated fish. Besides, results showed that only both pathogenic isolates interfered negatively with the *Isg15* stimulation triggered by poly I:C. These results suggest that the Isg15 might play an important role in host defense against RNA virus infection, and the pathogenic isolates used in this study may have mechanisms to evade or limit the Senegalese sole innate host defenses.

This study has beenfunded by the P09-CVI-4579 project, from Junta de Andalucía (Proyectos de Excelencia de la Junta de Andalucía) and AGL2014-54532-C2-1-R project, from Spanish Government.

CYTOKINE EXPRESSION WITHIN THE INTERBRANCHIAL LYMPHOID TISSUE OF ATLANTIC SALMON (*SALMO SALAR*) DURING AMOEBIC GILL DISEASE

C.C. NORTE DOS SANTOS[1]*, M.B. ADAMS[1], M.J. LEEF[1], B.F. NOWAK[1], S. BIRD[2]

[1]*IMAS Launceston, University of Tasmania, Australia*
[2]*University of Waikato, Hamilton, New Zealand*

Presence of Interbranchial lymphoid tissue (ILT) composed of intraepithelial lymphoid cell aggregations has been reported in the salmon gills. ILT is most likely part of the gill mucosal immune system, however its function is not fully understood. Histological studies have shown ILT is composed mainly of T cells supported by a meshwork of epithelial cells and it has been suggested that it could function as a secondary lymphoid organ. It is a unique tissue found in fish and it does not resemble any lymphoid tissues previously described in mammals. Increased the number of lymphocytes were observed in the ILT of Atlantic salmon affected by amoebic gill disease (AGD) 7 days after exposure to *Neoparamoeba perurans*. This was followed by an enlargement of the ILT and a decrease in the lymphocyte density at 14 and 28 days post exposure. Our current investigation further investigates the response of the ILT to AGD. This was done by measuring immune gene transcription within the ILT during AGD. Atlantic salmon were sampled prior to exposure and at 5 and 10 days post exposure to *N. perurans* trophozoites. Immune gene expression including selected cytokines relevant to innate and adaptive immune responses was done using Realtime-PCR. These results contribute towards our understanding of the role of the ILT in Atlantic salmon AGD.

BREEDING FOR AGD RESISTANCE IN ATLANTIC SALMON (*SALMO SALAR* L.) OF EUROPEAN ORIGIN

V. EMILSEN*, N. SANTI, J.S. TORGERSEN, J. ØDEGAARD, T. MOEN, S. KJØGLUM, S.A. KORSVOLL

AquaGen AS, Trondheim, Norway

Amoebic gill disease (AGD) caused by the amoeba *Paramoeba perurans* has been a major challenge for the salmon industry in Tasmania from the middle of the 1980's, and has in the last years been an increasing problem in Ireland, Scotland and Norway with reports of high mortalities. The disease is typically treated by immersing fish in freshwater baths (preferred) or hydrogen peroxide baths in closed tarpaulins or well boats. But repeated baths are often necessary, which is both a logistical and economical burden.

After 30 years of experience and studies with AGD, Tasmania has documented a substantial heritability for AGD resistance ($h^2 > 0.3$) in Atlantic salmon of North American origin. This suggests that improved resistance to the amoeba through selective breeding could contribute to permanent reduction of the problem. Resistance to AGD is associated with multiple genomic regions, and there are indications of a different mechanism of resistance in the first infection compared to all subsequent infections.

AquaGen has tested 100 families in an AGD challenge trial at VESO Vikan in 2014. The fish were bath challenged with *P. perurans*, and phenotypes as disease development (gill lesions) and mortality/survival of all individual fish were registered. The results showed a high heritability for survival ($h^{2=}0.55$) and a moderate heritability for gill score ($h^{2=}0.25$).

Siblings from the same families were also registered in a field trial with a natural outbreak of AGD. The heritability of gill score was 0.32 and there was a high genetic correlation between the field outbreak and the challenge trail.

The results demonstrate a considerable potential for improved resistance to AGD from selective breeding also in Atlantic salmon of European origin. A combination of traditional breeding combined with genomic selection would provide the fastest genetic improvement.

COMPARISON OF THE INNATE IMMUNE RESPONSE OF DIPLOID AND TRIPLOID ATLANTIC SALMON (*SALMO SALAR*) TO EXPERIMENTAL CHALLENGE WITH *NEOPARAMOEBA PERURANS*, CAUSATIVE AGENT OF AMOEBIC GILL DISEASE

L. CHALMERS[1]*, J. TAYLOR[1], W. ROY[2], A. PRESTON[2], H. MIGAUD[1], R.H. RICHARDS[1], A. ADAMS[1]

[1]*Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling, UK*
[2]*Marine Environment Research Laboratory, Machrihanish, UK*

The diversion of energy to gonad development during sexual maturation continues to cause problems for salmonid aquaculture. Sterile triploid fish have been proposed as a solution but to be considered commercially viable they must perform equally or better than diploids. This is especially important in response to disease outbreaks and, in particular, amoebic gill disease (AGD) which is now considered one of the most economically significant gill diseases of Atlantic salmon. The aim of this study was to compare the innate immune response of sibling triploid and diploid Atlantic salmon following an experimental infection with *Neoparamoeba perurans*, the causative agent of AGD.

Triplicate groups (n=60) of diploid and triploid Atlantic salmon smolts from two strains (A & B, n=30) were experimentally infected with *N. perurans* using pre-infected fish (n=6 tank^{-1}). Five fish per strain per tank were sampled at 7, 14, 21 and 28 days post-infection (d.p.i.). Gills were grossly scored for lesions and blood sampled for serum. Gills were processed and scored histologically according to Florent *et al*. (2009). Staining for mucous cells and immunohistochemistry for Na/K-ATPase was also undertaken. Lysozyme, complement and anti-protease activities were measured in 7, 14 and 21 d.p.i. serum samples.

The gill scores of infected fish were significantly higher than the respective uninfected group, regardless of ploidy. Histological scoring supported this, with infected fish gill pathology worsening over time. Assessment of Na/K-ATPase revealed migration of chloride cells to filament ends during infection. This was also noted for mucous cells, along with increased cell numbers. Lysozyme activity increased over time in diploids but decreased in triploids. At 14 and 21 d.p.i., diploids had significantly higher lysozyme activity than triploids. Anti-protease activity decreased in all ploidy groups from 7 to 14 d.p.i. then increased to 21 d.p.i. Significant ploidy differences were observed at all sampling points. For complement activity, variable patterns were observed between the groups, with no significant ploidy differences observed.

Gill score results suggest diploid and triploid Atlantic salmon were equally susceptible to infection by *N. perurans*, agreeing with previous research into parasitic infection (Frenzl *et al*., 2013). However, clear conclusions could not be drawn in terms of ploidy differences in immune competence due to the variability in immune assays results. As such, further work will be conducted. Overall, the findings enhance the current understanding of triploid Atlantic salmon immune function, suggesting that triploid Atlantic salmon are clinically as susceptible as diploids to AGD.

SUSCEPTIBILITY OF LUMPSUCKER, *CYCLOPTERUS LUMPUS*, TO INFECTION WITH *PARAMOEBA PERURANS*, AND SUBSEQUENT TRANSMISSION TO ATLANTIC SALMON, *SALMO SALAR* L., UNDER EXPERIMENTAL CONDITIONS

G. HAUGLAND[1], A.B. OLSEN[2], A. RØNNESETH[1], L. ANDERSEN[3]*

[1]*University of Bergen, Bergen, Norway*
[2]*The Norwegian Veterinary Institute, Bergen, Norway*
[3]*The Industrial and Aquatic Laboratory (ILAB), Bergen, Norway*

Cleaner fish such as lumpfish *Cyclopterus lumpus* L. and ballan wrasse, *Labrus bergylta* A. are increasingly used to delouse farmed Atlantic salmon, *Salmo salar* L. In 2013, more than 15 millions of cleaner fish were placed into net-pens with farmed salmon in Norway. Amoebic gill disease (AGD) caused by the parasitic amoeba *Paramoeba perurans* is an emerging disease condition of salmon farming in Northern Europe. The amoeba is ubiquitous and has been isolated from several fish species around the world, such as several salmonid species, turbot and wrasse. It has not been shown if lumpfish can be experimentally infected with *P. perurans,* and if so, if they will develop AGD and if they act as a vector for the spread of *P. perurans.* In the present study we show that lumpfish may be infected with *P. perurans* and develop AGD, but to a lesser extent than Atlantic salmon. We also show that lumpfish may act as carriers and transmit parasitic amoeba to Atlantic salmon. Moreover, we demonstrate the gill score lesion systems extensively used for evaluating AGD in Atlantic salmon, is less suitable for lumpfish infected with *P. perurans* as these may be non-symptomatic carriers.

PRACTICAL NUTRIENT RECOMMENDATIONS TO ENSURE ROBUST ATLANTIC SALMON (*SALMO SALAR*)

B.E. TORSTENSEN*, E.J. LOCK, N. SISSENER, E. HOLEN, S. REMØ, G.I. HEMRE, R. WAAGBØ

National Institute of Nutrition and seafood Research (NIFES), Norway

Atlantic salmon, as all animals, has specific nutrient requirements. Traditionally several of these nutrients were in surplus in fish oil and fishmeal. However, due the constant and limited supply of these marine raw materials, plant ingredients have increasingly replaced fish oil and fishmeal. Today more than 70% of farmed Atlantic salmon feed consists of plant ingredients, consequently changing the composition of a range of nutrients and other components. With these major changes in raw materials and hence nutrient composition, and possibly affecting the bioavailability of the nutrients, knowledge on practical nutrient recommendations when Atlantic salmon are fed plant-based diets, is pivotal.

The minimum requirement of a nutrient, such as specific amino acids, vitamins, minerals, fatty acids should be sufficient not only to sustain high growth rate, but also for the fast growing fish to be robust. Meaning that the nutrients in the diet should be provided at levels sufficient to ensure normal development, good welfare and fish health.

Increased lipid levels in Atlantic salmon liver is an indicator of nutrient deficiency causing metabolic imbalance, which may lead to increased inflammation and decreased robustness of the fish. In the EU-project Arraina, Atlantic salmon was fed increasing levels of a nutrient package containing a mix of minerals, vitamins, amino acids, cholesterol at two life stages (in fresh water and in sea water). At both life stages, the current nutrient requirement levels (NRC 2011) were not sufficient to prevent lipid accumulation in Atlantic salmon livers. Increased liver lipid is a general biomarker, and further analyses of biomarkers for inflammation, oxidation status and stress, and including metabolic profiling, is necessary to determine the minimum nutrient requirement for robust Atlantic salmon. Results linking practical nutrient requirements with fish metabolism, welfare and health representing production of robust Atlantic salmon will be presented and discussed.

EFFECTS OF DIETARY LIPIDS ON CORTISOL METABOLISM AND RESPONSE OF GLUCOCORTICOID RECEPTORS: A REVIEW

D. MONTERO[1]*, M.J. CABALLERO [1], G. TEROVA[2], S. RIMOLDI[2], S. TORRECILLAS[1], L. TORT[3], M.S. IZQUIERDO[1]

[1]*Grupo de Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain*
[2]*University of Insubria, Varese, Italy*
[3]*Universitat Autonoma de Barcelona, Barcelona, Spain*

The changes in circulating plasma cortisol have been traditionally used as an indicator of stress in fish, although less information is available about specific mechanisms involved in the stress response such as the recognition of the stressful situation or the cortisol-mediated response of glucocorticoid receptors (GR) within the different tissue, determining all of them the ability of the whole organism to cope with the stressful situation.
Dietary lipids has been widely described on the capacity of fish to adapt to stressful situations, altering circulating levels of plasma cortisol, but little is known on the mechanisms on how dietary lipids modulate the different processes involved in the cortisol metabolism. Dietary lipids also have a regulatory role on the synthesis and release of cortisol from the interrenal cells, and have been also showed to have a modulatory effect on the cell response to increased levels of cortisol, mediated by the GRs.
The type of lipid in diet formulae is continuously changing due to the strategies to substitute fish oils by blend of different vegetable oils in aquaculture diets. Thus, it is important to focus new studies on the role of dietary lipids on the stress-related changes in target tissues responsible of important processes such as the production of mucus, mobilization of energy reserves or even the immune system.
Studies on the role of dietary lipids in fish stress response will be reviewed, including those related with the metabolic routes implied in the cortisol secretion from interrenal cells, and those that focus on the modulate role of certain fatty acids on the interaction between GR and some transcription factors at expression of genes level.

EFFECT OF NUTRITIONAL INTERVENTIONS ON IMMUNE RESPONSE AND GUT HEALTH OF GILTHEAD SEA BREAM (*SPARUS AURATA*)

A. SITJÀ-BOBADILLA[1]*, I. ESTENSORO[1], J. CALDUCH-GINER[1], B. FOUZ[2], C.L. BROWDY[3], A. ORTIZ[4], J. PÉREZ-SÁNCHEZ[1]

[1]*Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), Castellón, Spain*
[2]*University of Valencia, Valencia, Spain*
[3]*Novus International, Inc., St. Charles, MO, USA*
[4]*Norel S.A., Madrid, Spain*

Aquaculture sustainability of most Mediterranean fish species relies on plant ingredients as an alternative to fish protein and fish oil (FO) for aquafeeds, but the detrimental health effects can limit replacement levels. Feed additives have been proposed as an alternative to mitigate negative effects of anti-nutritional factors and extend opportunities for replacement of ingredients from finite marine resources. We compile here the results of a series of studies with different feed additives in juvenile gilthead sea bream (GSB). The additives tested were: 1) a sodium butyrate preparation (BP-70®Norel) and 2) a combination of the essential oils carvacrol and thymol, NEXT ENHANCE®150 (Novus, NE), with or without a prebiotic (Previda®, PRE). The effects of BP-70 added at 0.4% to a diet in which FO was replaced at 84% by a blend of vegetable oils (VOs), were compared with a control diet with no FO replacement, and another diet replacing VOs at 58%. NE was given at 100 ppm alone or in combination with PRE (0.5%) and compared to a control diet. For both products, gut health was evaluated histologically, immunocytochemically and at the transcriptomic level with a 96-well PCR array for 86 genes, including markers of: cell differentiation and proliferation, intestinal architecture and permeability, enterocyte mass/function and epithelial damage, immune-surveillance (interleukins, cytokines and chemokines receptors, pattern recognition receptors), and mitochondria function and biogenesis. For BP-70 (added at 0.8%), the effects on innate humoral parameters, intestinal microbiota and survival after a challenge with *Photobacterium damselae* subsp. *piscicida* were also evaluated. The expression profile in fish fed the control and the low substitution diet was almost equal, whereas a wide range of markers including those of cell proliferation and differentiation, intestinal barrier function antioxidant defence, interleukin function and lectin recognition system were overexpressed in the extreme diet fish. However, this expression pattern was almost reversed to the gene expression profile of control fish with butyrate supplementation. Diets with NE and/or PRE provoked significant changes in the expression of 26 genes, inducing an anti-inflammatory and anti-proliferative transcriptomic profile. An apparent benefit of BP-70 inclusion in terms of stimulation of the innate immune response and protection against photobacteriosis was also observed. The lower microbial diversity observed in BP-70 fed fish could indicate that their intestinal microbiota achieves a stable situation faster than that from control animals. Butyrate supplementation is a promising strategy for GSB, especially when fish are facing stress associated with sustainable diets with extremely low inclusion levels of fish meal and FO. The dietary combination of NE and PRE could have a potential use for overcoming some types of nutritionally or pathologically induced gut inflammation.

EFFECTS ON BONE MORPHOLOGY OF *SPARUS AURATA* FED DIETS HIGH IN VEGETABLE INGREDIENTS WITH DIFFERENT MICRONUTRIENT LEVELS

D. DOMÍNGUEZ[1]*, D. MONTERO[1], L.E. ROBAINA[1], K. HAMRE[2], V. KARALAZOS[3], M.S. IZQUIERDO[1]

[1]*Grupo de Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain*
[2]*National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway*
[3]*Biomar UK, Grangemouth, Scotland*

Substitution of marine ingredients (namely fish meal and oil) to lower the price of feeds is a common practice. However this replacement leads to a change in the micronutrient profile of the feed which may translate into a reduction in productive parameters and an alteration of the fish's health status. The addition of micronutrients to the diet in optimal concentrations may solve these problems, however few in depth studies on micronutrient requirements have been conducted on *Sparus aurata*, thus trace elements addition in feeds may not be covering the species requirements. Bone tissue is mainly composed by a mineral matrix, making it especially susceptible to these changes, and suboptimal nutrients levels usually translate into changes in its morphology.

In this study six different isoenergetic and isonitrogenous practical diets containing a premix of the objective trace elements in ascending order including the current standard (0%, 25%, 50%, 100%, 200%, 400%), and a + control diet based on fish meal (FM) were tested. The complete nutrient package included Selenium, Iodine, Copper, Cobalt, Manganese, Iron, Zinc, Calcium, Thiamin, Riboflavin, Niacin, Pantothenic acid, Piridoxin, Biotin, Folic acid, Cyanocobalamin, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin C, Taurine, Methionine, Histidine and Cholesterol. The levels on the 100% nutrient package were theoretically based on the identification of nutrients required to avoid reduced performance and health status in sea bream when fed high levels of plant products.

Two thousand six hundred and twenty five gilthead sea bream (Sparus aurata) juveniles were randomly located into twenty-one 500 l fiberglass tanks. Fish average initial weight was 25.45 ± 2.74 g (mean ± S.D.). Fish were manually fed until apparent satiation three times per day. Each diet was tested in triplicates during 5 months, when fish body weight was over 100 g. For the final sampling 20 fish per tank were collected for radiographic study where bone anomalies were analyzed according to Boglione et al., (2001) protocol.

Fish growth increased with increasing levels of micronutrients up to 50% inclusion. Results demonstrated a decrease in the number of fish affected by skeletal anomalies with increasing minerals and vitamins concentrations. Different vertebral morphologies were described for the different diets.

REDUCTION OF BONE ANOMALIES INCIDENCES IN GILTHEAD SEABREAM LARVAE FED NUTRITIONALLY IMPROVED MICRODIETS

M.S. IZQUIERDO*, D. DOMÍNGUEZ, C.M. HERNÁNDEZ-CRUZ, R. SALEH

Grupo de Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain

Morphological anomalies, particularly the skeletal malformations affecting to the opercula complex, neurocranium or vertebral column, still have a considerable negative economical incidence in marine fish production. Several genetic and epigenetic factors have been linked to skeletal anomalies in cultured teleost fish, including nutritional factors. Several vitamins, minerals and lipid nutrients influence the occurrence of skeletal malformations. These nutrients are particularly important for the normal osteological development of fish larvae and appearance of deformities such as vertebral fusion can be detected as early as 20 dah. Therefore, feeding of marine fish larvae during early development may determine later on life the incidence of skeletal deformities. The development of early weaning diets, as a delivery factor for nutrients during larval development, is one of the objectives of the ARRAINA project, aiming to produce healthy and high quality juveniles with reduce incidences of malformation. For that aim, several levels, ratios and types of nutrients were fed to gilthead seabream and their effects on larval survival, anomalies occurrence, mineralization and expression of several molecular markers of bone development were studied. The results showed that nutrients such as Zn, Mn, Se, DHA or phospholipids levels markedly affect cartilage and bone development in this species as well as the percentages of different types of skeletal malformations.

EFFECTS OF COMPLETE REPLACEMENT OF FISH MEAL AND FISH OIL BY VEGETABLE MEALS AND OIL ON HEALTH AND DISEASE RESISTANCE OF EUROPEAN SEA BASS *DICENTRARCHUS LABRAX*

S. TORRECILLAS[1]*, D. MOMPEL[1], M.J. CABALLERO[1], D. MONTERO[1], L. ROBAINA[1], V. KARALAZOS[2], S. KAUSHIK[3], M.S. IZQUIERDO[1]

[1]*Grupo de Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Canary Islands, Spain*
[2]*Biomar UK, North Shore Road, Grangemouth FK3 8UL, Scotland*
[3]*INRA-UMR Nutrition Aquaculture Génomique, Pôle Hydrobiologie, 64310 Saint Pée-sur-Nivelle, France*

The main objective of this study included in the ARRAINA project is to evaluate the effects of graded and combined replacement of FO and FM by terrestrial meals and oils on health and disease resistance of European sea bass (*Dicentrarchus labrax*). For that purpose, European sea bass juveniles of 9.8± 1.5 g and 9.1± 0.5 cm were distributed in 27 cylindroconical 500 L tanks at an initial stocking density of 1.8 $kg \cdot m^{-3}$ and fed during 88 days nine isoproteic (45%) and isolipidic (21%) diets containing graded and combined levels of FO and FM as follows: B1 (58%FM/15%FO), B2 (20%FM/6%FO), B3 (20%FM/3%FO), B4 (10%FM/6%FO), B5 (10%FM/3%FO), B6 (5%FM/6%FO), B7 (5%FM/3%FO), B8 (0%FM/0+% FO) and B9 (0% FM/0%FO). After 12 weeks of feeding, fish were sampled growth performance, intestinal morphometry and mucus production and microbiota profiles studies. Besides, fish fed B2, B3, B6 and B7 diets were subjected to a short-term in vivo and in vitro exposure to *Vibrio anguillarum* via anal canalization. Fish fed FM levels of 58-20% presented the highest ($P<0.05$) SGR, whereas fish fed 0%FM without LC-PUFA supplementation presented the lowest ($P<0.05$). Besides, fish fed a 20%FM did not differ in SGR from those fed a 10%FM and fish fed a 10%FM from those fed a 5%FM. No effect of FM and FO levels were found for final survival and ISI. Simple main effects analyses showed a clear influence of lipid content on intestinal "*ex vivo*" translocation percentages. Indeed, "*in vivo*" intestinal translocation in terms of final survival followed a similar trend. Mucosal folds height, width were not affected by FM or FO dietary levels when related to the real fish weight. Reduced levels of dietary FO increased anterior gut lamina propria width and lower dietary FM levels resulted in engrossed posterior gut submucosa. Mucus production was neither affected by FM or FO percentages, however fish fed the lower levels of FM/FO presented the higher number of goblet cells by unit of area. GALT-related gene expression and microbiota profiles were also correlated with the dietary level of FM and FO.

INFLUENCE OF FEED LIPID SOURCE ON KIDNEY MACROPHAGE RESPONSE IN TILAPIA (*OREOCHROMIS NILOTICUS* L.)

J.A. GARCÍA[1]*, M. VILLARROEL[2]

[1]*Univ. Complutense de Madrid, Avda, Puerta de Hierro s/n, 2804-Madrid*
[2]*Univ. Politécnica de Madrid, Avda, Complutense s/n, 28040-Madrid*

Traditionally, fish feeds have depended on fish oil as energy source and essential fat acids. However, the strong demand on fish oils for both human and animal consumption has led to a significant increase in its price, and to a decrease in the available quantities of this product. This fact has forced the search for new lipid sources for fish feed, and different vegetable oils have been studied as alternatives for fish oil. But the use of these vegetable oils could lead to metabolic disturbances that could end in an immune response dysfunction. Three different feeds differing only in the plant oil included (linseed, sunflower oil and high oleic sunflower oil) were used to feed tilapia during 2 months. A fourth group fed with a feed with fish oil was included as control group. After that time, kidney macrophages were collected and their capacity to control and eliminate a bacterial infection was tested by counting the number of macrophages, and estimating the number of bacteria/macrophage at 0 and 4 hours post-infection. Respiratory burst was also recorded as a measure of macrophage function. Feeds were formulated to fulfil fish requirements, and weight increase and conversion rate were very similar at the end of the experiment in the four groups. Results showed a higher survival of macrophages to the bacterial infection with sunflower oil, although macrophages from the group of high oleic sunflower oil showed similar respiratory burst results with a lower number of surviving macrophages. Fish from the group fed with linseed oil showed the lowest surviving macrophage rate, while the lowest result in respiratory burst was achieved in the group fed fish oil. However, these two groups showed similar bacterial counts per macrophage.

References:
Watanabe, T. 2002. Strategies for further development of aquatic feeds. Fisheries Science, 68: 242-253.
Yu-Hung Lin y Shi-Yen Shiau. 2003. Dietary lipid requirement of grouper, Epinephelus malabaricus, and effects on immune responses. Aquaculture, 225: 243-250.
Montero, D., T. Kalinowski, A. Obach, L. Robaina, L. Tort, M.J. Caballero y M.S. Izquierdo. 2003. Vegetable lípido sources for gilthead seabream (Sparus aurata): effects on fish health. Aquaculture, 225: 353-370.
Montero, D., V. Grasso, M.S. Izquierdo, R. Ganga, F. Real, L. Tort, M.J. Caballero y F. Acosta. 2008. Total substitution of fish oil by vegetable oils in gilthead seabream (Sparus aurata) diets: Effects on hepatic Mx expression and some immune parameters. Fish and Shellfish Immunology, 24: 147-155.
García, J.A. y M. Villarroel. 2009. Effect of feed type and feeding frequency on macrophage functions in tilapia (Oreochromis niloticus L.). Fish and Shellfish Immunology, 27: 325-329.

MICROBIAL FEED ADDITIVE ALLEVIATES INTESTINAL INFLAMMATION IN ATLANTIC SALMON

V. KIRON*, G.K. VASANTH, A. KULKARNI, D. DAHLE, J. LOKESH, Y. KITANI

University of Nordland, Bodø, Norway

Feed-linked intestinal inflammation in Atlantic salmon is widely recognized as a welfare issue of the farmed fish. Adequate prophylactic measures need to be developed to counter gut disorders and improve fish health. Gut microbes are known to safeguard the intestinal mucosa and it is hypothesized that in-feed delivery of microorganisms could help alleviate inflammatory situations. Here, the efficacy of Bactocell®, an approved microbial feed additive, in countering intestinal inflammation in Atlantic salmon was evaluated in two groups of fish – one offered a commercial feed top-coated with Bactocell® (microbial group) and another offered the feed without the additive (control group) for 6-weeks. Distal intestinal inflammation, chemically induced by oxazolone intubation, was marked by prominent changes in tissue micromorphology and expression of inflammation-related genes in both groups only at 24 h, based on assessments at 3 h, 24 h and 3 weeks after inducing inflammation. At 24 h, the intestine of the control group had severe oedema, widening of intestinal folds and lamina propria, dislocated enterocytes and goblet cells, infiltration of granulocytes, and higher levels of the marker genes, compared to the microbial group or other time points of the control group. At 24 h, the altered proteins in the microbial group, compared to the control group, were Calr, Trp1, Psma2, Naga and Catsb. These molecules could be aiding the protective mechanisms against inflammation in the intestinal mucosa. The speed of recovery was also different by the third week after intubation – the inflammatory responses did not persist in the microbial group unlike the control group. The findings indicate that Bactocell® helps to counter inflammation and contributes to intestinal homeostasis in Atlantic salmon.

FUNCTIONAL FEEDS WITH KRILL MEAL REDUCE HEART PATHOLOGY AND VIRAL LOAD IN HEART AND SPLEEN OF ATLANTIC SALMON (*SALMO SALAR*) AFTER EXPERIMENTALLY INDUCED INFECTION WITH PISCINE REOVIRUS (PRV)

T. SIGHOLT[1]*, Ø. EVENSEN[2], C. WALLACE[3]

[1]*BioMar AS, Trondheim, Norway*
[2]*Norwegian University of Life Sciences (NMBU), Oslo, Norway*
[3]*VESO Vikan, Namsos, Norway*

Heart and skeletal muscle inflammation (HSMI) is characterised by epi-, endo- and myocarditis, myocardial necrosis, and myocytis and necrosis in skeletal muscle. HSMI usually occurs 5-9 months after seawater transfer of Atlantic salmon (*Salmo salar*). Mortality may vary from 0% up to 20%. The disease is associated with *Piscine reovirus* (PRV), a virus that replicates at early stages in the erythrocytes of Atlantic salmon (*Salmo salar*). Totally 181 cases of HSMI was registered in 2014 in Norway.

In the present study, the effects of a functional feed containing meal from Antarctic krill (*Euphasia superba*) were compared to a copy of commercial feed. Both control and test feed had similar levels of lipid, protein and sum of EPA and DHA, but different levels of marine phospholipids provided by krill meal.

Fish were fed the control and test diets in fresh water over 8 weeks before seawater transfer and during the 14 weeks challenge period in sea water. Shedders (i.p. injected with PRV-containing tissue homogenate) were transferred to the tanks at seawater transfer (weeks post challenge 0).

PRV was measured with RT-qPCR in heart and spleen, and histopathological changes were evaluated with a visual scoring index in heart, liver, spleen and head kidney 0, 6, 8,9,10,12 and 14 weeks post infection (wpc).

Fish weight increased from a mean weight of 19.6 g at start to 227 g in the control and 269 g in the test group during the trial.

The histopathological changes were largest in wpc 8 in the spleen and head-kidney and in wpc 10 in the heart ventricle tissue. A significant lower virus load was found in the heart and spleen in both wpc 6 and 8 compared to the control group. There was a significant lower change in histopathology score in wpc 8 in the head kidney and in wpc 10 in the heart ventricle tissue in the test group compared to the control group.

The results showed that a functional feed with krill meal can delay or reduce the replication of PRV and mitigate the severity of HSMI.

TRANSCRIPTOMIC RESPONSE TO A FUNCTIONAL FEED DURING PISCINE REOVIRUS (PRV) INFECTION IN ATLANTIC SALMON (*SALMO SALAR*).

Z. HEIDARI[1], J. TINSLEY[2]*, R. BICKERDIKE[2], T. SIGHOLT[3], A. DOUGLAS[1], J. ZOU[1], Ø. EVENSEN[4], C. WALLACE[5], S.A.M. MARTIN[1]

[1]*University of Aberdeen, Aberdeen, UK*
[2]*BioMar Ltd, Grangemouth, UK*
[3]*BioMar AS, Trondheim, Norway*
[4]*Norwegian University of Life Sciences (NMBU), Oslo, Norway*
[5]*VESO Vikan, Namsos, Norway*

Heart skeletal muscle infection (HSMI) is an important disease of farmed Atlantic salmon (*Salmo salar*). The causative agent is *Piscine reovirus* (PRV) which belongs within the family *Reoviridae*. The disease was first detected in Norway 1999 but is now present in Scotland & Ireland. Whilst reports vary, mortality can reach 20% in specific cases. Clinical symptoms of the disease are described as epi-, endo- and myocarditis, myocardial necrosis, myocytis and necrosis in the red muscle. Due to its economical significance functional feeds designed to mitigate against the effects of the disease are required by the industry. Functional feeds are a form of clinical nutrition where a given component is enriched beyond the dietary requirement in order to benefit health or reduce severity of disease.

In this study a cohabitation trial was carried out to compare the effects of a functional feed containing Antarctic krill (*Euphasia superba*) to a commercial feed. Transcriptome analysis in the heart tissue at 6, 8 and 10 weeks post infection was carried out using a 44k microarray. Viral load was quantified in the ventricle using RT-PCR. Histopathology within the heart tissue was assessed using an established semi-quantitative methodology.

Microarray analysis demonstrated that a total of 710 genes were differentially regulated in the functional feed compared to the control. 480 genes of these genes were differentially higher expressed and 230 were lower expressed in the functional feed compared to the control diet during the peak of infection. The peak of viral infection was determined to be 8 weeks post infection. A number of biological processes in the transcription of genes such as immune response, defence response, generation of precursor metabolites and energy as well as carbohydrate catabolism and cell chemotaxis were positively effected by the functional feed.

The results demonstrate that a functional feed can reduce the severity of the disease in infected fish. Further work is required to understand the mechanisms behind this improvement. Ultimately fish, which have an improved response during the disease and recover quicker will suffer from reduced mortality and improved growth for the benefit of fish welfare and increase production of farmed salmon.

MONITORING OF ENVIRONMENTAL HEAVY METALS ACCUMULATION IN FISH MUSCLES FROM THE TWO LAKES IN MLADENOVAC MUNICIPALITY, BELGRADE AREA, SERBIA

B. PETRUJKIĆ[1], D. JOVANOVIĆ[1], R. MARKOVIĆ[1], J. JANJIĆ[1], D. JAKIĆ-DIMIĆ[2], V. TEODOROVIĆ[1], D. ŠEFER[1]*

[1]*Faculty of Veterinary Medicine, University of Belgrade, Serbia*
[2]*Scientific Veterinary Institute, Belgrade, Serbia*

The major routes of heavy metal uptake by man are food, water and air. Namely fish, are the most important source of mercury and arsenic for human beings. In order to evaluate the current state of heavy metal accumulation in fish, heavy metal content of fish muscles in the two lakes of Mladenovac Municipality, Belgrade area, Serbia was analyzed.
The samples of the Common carp (*Cyprinus carpio*), Pike fish (*Esox lucius*) and Crusian carp (*Carassius auratus gibelio*) were taken on to the locations: Rabrovac Lake and Markovac Lake, during late August 2013. The content of Pb, Cd, Hg, As, Cu, Fe and Zn was determined in the fish dorsal muscle by atomic absorption spectrophotometer (AAS) (Perkin-Elmer Analyst 700).
The lowest concentration of lead in fish muscles was observed in Common Carp and highest in Pike fish, both sampled in Rabrovac Lake ($P<0.01$). The content of mercury has shown the highest variation between the tested fish species ($P<0.01$) and ranged between 0.119±0.008 mg/kg in Crusian carp (Markovac Lake) and 0.401±0.011 mg/kg in Common carp (Rabrovac Lake). The concentration of arsenic was higher in Common carp ($P<0.01$), than in Pike fish and Crusian carp. The concentrations of the cadmium, iron and zinc determined in our study have shown the slightest variation between sampled fish species.
Our survey determined that none of the investigated metals in fish muscles exceeded the Maximum Levels (MLs) set by European regulations in muscle tissues (1881/2006/EC and 629/2008/EC). The maximum levels set by the Serbian Rule Book (2002) were not exceeded either. Based on the results of investigating the heavy metal content of fish samples, measured in our study, fish meat from Rabrovac and Markovac Lakes can be regarded as safe. Therefore the use of these fish in human nutrition poses no risk to the potential consumers.

THE RESULTS OF THE FIRST NATIONWIDE SURVEY ANALYSING CAUSES AND PREDISPOSING FACTORS FOR LOSS OF FISH TROUGHOUT THE ON-GROWING PERIOD AT SEA IN NORWEGIAN SALMONID AQUACULTURE

H. BLEIE[1]*, A. SKRUDLAND[2], M. STORMOEN[3]

[1]MSD Animal Health, Thormölensgt. 55, 5008 Bergen, Norway
[2]Mattilsynet, PO Box 383, 2381 Brumunddal, Norway
[3]Marine Harvest, PO Box 4102, 5835 Bergen, Norway

A nationwide survey covering the generations of Atlantic salmon *(Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) transferred to sea in the autumn of 2010, spring of 2011 and autumn of 2011 was carries out retrospectively subsequent to harvested and hence as the end point biological data were available. The production data were retrieved by questionnaires being filled in by site managers and returned to the project manager. One questionnaire was filled in for each group of fish, which was defined as uniform smolt from one smolt plant transferred to a specific sea site during a short time interval. A total of 1.066 groups were covered, representing 318 sea sites, 59 proprietors with stocks from 139 smolt plants. A total of 307 million fish were included in the study. Data documenting causes of loss of fish were separated in three consecutive time intervals; from stocking and through the third month at sea, from the fourth month to the end of the 10th month, and from the 11th month to harvest. The absolute numbers of fish registered lost were given and further divided in to categories of loss and specific causes of loss. All data were retrieved from the database of biomass recordings maintained by all fish farmers in Norway. Data were plotted anonymously into an Excel spreadsheet and transferred to STATA 12© for epidemiological analysis. The data collection and project management was carried out by Mattilsynet (Norwegian Food Safety Authority) and the statistical analysis done at the Centre for Epidemiology and Biostatistics at the Faculty of Veterinary Medicine and Bioscience within the Norwegian University of Life Sciences (NMBU). The project was financed by The Norwegian Seafood Research Fund (FHF). A whole range of factors like geographical differences, management regimes and biological strategies were disclosed as predisposing for the loss of fish and will be presented.

DISEASE MANAGEMENT MITIGATES RISK OF PATHOGEN TRANSMISSION FROM FARMED SALMON

S.R.M. JONES[1,*], D.W. BRUNO[2], L. MADSEN[3], E.J. PEELER[4]

[1]*Pacific Biological Station, Nanaimo, Canada*
[2]*Marine Scotland Science, Aberdeen, Scotland*
[3]*Technical University of Denmark, Frederiksberg, Denmark*
[4]*Cefas, Weymouth, United Kingdom*

Open marine net pens facilitate virus and sea lice transfer occasionally leading to infections and outbreaks of disease in farmed salmon. A review of three salmon diseases (infectious salmon anaemia, pancreas disease and salmon lice) shows that increased risk of exposure to neighbouring farms is negatively related to distance from and positively related to biomass at the source of infection. Epidemiological techniques integrating data from oceanography, diagnostics, pathogen shedding rates and viability contribute to improved understanding of pathogen transmission pathways among farms and permit the designation of areas of risk associated with sources of infection. Occupation of an area of risk by susceptible fish may increase the likelihood of exposure, infection and disease. Disease mitigation in mariculture occurs at two scales: area-based (coordinated stocking, harvesting and fallowing) and farm-based (vaccination, early pathogen detection, veterinary prescribed treatments and depopulation or early harvest in the event of viral disease). Collectively, implementation of mitigation measures results in virus disease outbreaks of shorter duration with lower mortality and therefore reduce the likelihood of pathogen transmission. In contrast, the mitigation of sea lice transmission is less likely to be effective in some areas due to the loss of parasite sensitivity to therapeutants and to the absence of treatment when parasites occur below management thresholds. Risk of pathogen spillback to wild populations is estimated from epidemiological data however validation efforts using direct surveillance of wild populations require ongoing commitment.

OUTCOMES OF 30 YEARS FISH DISEASE CONTROL IN GERMANY

D.W. KLEINGELD*, A. WELZEL

Lower Saxony State Office for Consumer Protection and Food Safety, Veterinary Task Force, Hannover, Germany

In Germany regulations on the control of certain fish diseases are in force for over 30 years. Compared to the control of terrestrial animal diseases, the control of fish diseases faces special epidemiological challenges. Moreover the pertinent legislation on fish disease control is currently subject of very controversial discussions in Germany.
In retrospect however, achievements of fish disease control measures in Germany can be confirmed. The number of zones or compartments declared free of Viral Haemorrhagic Septicemia (VHS) and Infectious Haematopoietic Necrosis (IHN) has increased significantly. Moreover the whole territory of Germany is considered to be free of Infectious Salmon Anaemia (ISA). In the course of the implementation of Directive 2006/88/EC, the number of IHN and VHS-free compartments and zones has also increased in other EU Member States. Regarding Koi Herpes Virus Disease (KHVD) first zones and compartments have been declared free within the EU.
According to official surveys, the incidence of VHS and IHN is declining in Germany since 1996. Also the KHV-I incidence has dropped significantly since 2007. Although the incidence doesn`t allow reliable statements regarding epidemiology, this development must be considered as a success inter alia due to fish disease control measures. Furthermore the awareness regarding biosecurity of both aquaculture production business operators and ornamental fish keepers was heightened since the fish disease control measures came into force. Finally eradication measures in the course of disease outbreaks turned out to be economically profitable at least in case of salmonid diseases.
Statistical and epidemiological data on outbreaks of notifiable and reportable fish diseases are officially collected in the German Animal Diseases Reporting System (TSN). From TSN detailed information on primary outbreaks of notifiable fish diseases are sent via the Animal Disease Notification System to both the EU-Commission and the other Member States within 24 hours. In case of secondary outbreaks the Commission must be notified via ADNS within one week. In Germany the Animal Disease Information System (TSIS) provides information on outbreaks of notifiable animal diseases since 2014 also for the public.
The obligation that aquaculture production business operators have to conduct preventive measures according to the German Animal Health Act will certainly have a positive impact regarding the objectives of the fish disease control legislation. Prevention is also an important element of the Animal Health Strategy of the European Union. The future EU Animal Health Law will have an impact on fish disease control. The regulation will certainly contribute to the harmonization of fish disease control measures within the EU.
Finally and also for competitive reasons, fish disease control measures should be implemented consequently throughout the EU.

SPACE-TIME MODELLING OF THE SPREAD OF PANCREAS DISEASE WITHIN AND BETWEEN NORWEGIAN MARINE SALMONID FARMS

M. ALDRIN[1]*, R.B. HUSEBY[1], P.A. JANSEN[2]

[1]*Norwegian Computing Centre, Oslo Norway*
[1]*Norwegian Veterinary Institute, Oslo, Norway*

Infectious diseases are a constant threat to industrialised farming, which is characterised by high densities of farms and farm animals. Several mathematical and statistical models on spatio-temporal dynamics of infectious diseases in farmed host populations have been developed during the last decades, covering various farming systems. Here we present such a spatio-temporal stochastic model for the spread of a disease between and within aquaculture farms. The spread between farms is divided into several transmission routes, including i) distance related to spread between neighbouring farms and ii) between farms through other types of contagious contacts. The within-farm infection dynamics is modelled by a susceptible-infected-recovered (SIR) model. We apply this framework to model the spread of pancreas disease (PD) in marine farms in Norway, using data covering all farms producing salmonids over 9 years. The motivation for this exercise is partly to gain insight into the spatio-temporal dynamics of this disease in salmonid farming, which is of importance to disease control. We find, for example, that the within-farm infection pressure varies with season and we estimate the timing from unobserved infection events to disease outbreaks are detected. Partly our motivation was also to use the model for scenario simulation to test the effect of various control strategies. The simulations suggest that if a culling strategy is implemented, with culling of infected fish stocks within a month post PD detection, the number of detected disease outbreaks per year may be reduced by 57 % after the full effect has been reached.

MOLECULAR TRACING CONFIRMS THAT INFECTION WITH INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) FOLLOWS THE SMOLT FROM HATCHERY TO GROW OUT FARM

A.B. KRISTOFFERSEN*, B. BANG JENSEN, V. ASPEHAUG, O. GJELSTENLI, O. BRECK, M. DEVOLD

Norwegian Veterinary Institute, Oslo, Norway
PatoGen Analyse AS, Ålesund, Norway
Marine Harvest ASA

Infectious Pancreatic Necrosis (IPN) is one of the most important virus diseases in European salmonid aquaculture. The virus can be transmitted both horizontally and vertically, and was originally a problem in juvenile fish in freshwater production, but has since the 1980'es been causing trouble in the marine phases of salmonid production. It is believed, but has not been proved, that smolt infected in the hatchery can carry the virus with them to the marine phase, where it can then be reactivated.

In Norway, the number of IPN cases has been declining in recent years, while the situation in the rest of Europe is stable. Fighting the virus in Norway has been approached by two different strategies, either by marker-assisted breeding fish that are more resistant towards IPNV (So-called QTL-fish) or by sanitizing the hatcheries to get rid of IPNV 'house-strains'. We have obtained data from a Norwegian company that over several years have used the sanitizing strategy. This company experiences the same decline in IPNV cases as the other companies. The data includes screening data and sequence information from hatcheries and marine grow out farms from 2010 to 2013. We have looked into three different questions:

1. Are IPNV-sequences obtained from the same fish groups in hatcheries and marine grow out sites more similar than could be expected if the virus was not carried from the hatchery to the sea?
2. Is the similarity between IPNV-sequences from a marine farm and the hatchery that supplied the smolt significantly higher than between sequences from a marine farm and any hatchery?
3. Are IPNV-sequences more similar within hatchery than between hatcheries? (confirming the existence of 'house-strains')

The answers to all these questions were yes. Thus, we have validated that IPNV is carried by the smolt from hatchery to the sea, and the existence of hatchery-specific house-strains that are repeatedly infecting successive fish groups within the hatchery by the use of robust, statistical methods.

RISK FACTORS FOR OUTBREAKS OF INFECTIOUS PANCREATIC NECROSIS (IPN) AND FOR MORTALITY IN NORWEGIAN SALMONID POSTSMOLTS

B. BANG JENSEN*, A.B. KRISTOFFERSEN

Norwegian Veterinary Institute, Oslo, Norway

Infectious Pancreatic Necrosis (IPN) has for many years been considered one of the most important restraints to the production of salmonids in European aquaculture. In Norway, the disease is responsible for high losses in post-smolts in the first few weeks after sea-transfer. Despite the importance of IPN, there are few epidemiological studies on risk factors and mitigation strategies. Here we present analyses of risk factors from data from all cohorts put to sea in 2009-2012 on Norwegian marine salmonid farms. The variables included were obtained from national registers on salmonid production and disease outbreaks.

We performed a univariate linear regression with cumulative mortality over the first six months after sea transfer to describe the influence of different variables on mortality. Subsequently, a multivariate logistic model was developed and used to identify significant risk factors for IPN-outbreak.

The results showed that the risk of IPN outbreak was higher for spring vs autumn cohorts, Atlantic salmon vs. rainbow trout and for cohorts on farms with previous history of IPN. The risk increased with increasing cohort size and infection pressure, whereas increasing temperature and weight at sea-transfer decreased the risk.

Estimations from a model of cumulative mortality within the first six months after sea-transfer showed that mortality in cohorts with IPN increased to approximately 7.2% as compared to a "baseline" cohort with a mortality of 3.4%. If the cohort had both IPN and PD, the estimated mortality increased to 12.9%, and cohorts with both IPN, PD and HSMI had an estimated mortality of 16.6%, when all other significant factors were kept constant (cohort type=spring, year=2012, temperature at sea-transfer=9,92^{o}C and weight at sea-transfer=107g).

Our results provide valuable inputs for mitigation strategies and for economic modelling of consequences of disease.

GENETICS OF VHSV IN EUROPE

M. CIESLAK[1], M. BAUD[2], N. DISERENS[3], M. ENGELSMA[4], O. HAENEN[4], S. MOUSAKHANI[1], N.J. OLESEN[5], V. PANZARIN[6], H.F. SKALL[7], T. WAHLI[3], H. SCHÜTZE[1]*

[1]*Friedrich-Loeffer-Institute, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany*
[2]*ANSES Technicienne Biologie Moléculaire, Unité de Pathologie Virale des Poissons, Ploufragan/ Plouzané, France*
[3]*Centre for Fish and Wildlife Health, University of Bern, Switzerland*
[4]*Central Veterinary Institute of Wageningen, Lelystad, The Netherlands*
[5]*Technical University of Denmark, National Veterinary Institute, Denmark*
[6]*Research & Innovation – Department of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie, Italy*
[7]*Aarhus University Faculty of Agricultural Sciences, Denmark*

Viral hemorrhagic septicemia (VHS) plays an important role in aquaculture. The notifiable disease causes high significant economic losses due to high mortality especially in farmed rainbow trout (*Oncorhynchusmykiss*), turbot (*Scophthalmusmaximus*), and Japanese olive flounder (*Paralichthysolivaceus*). Susceptible species are salmonids. Until now, more than 80 affected species were reported. However, in European aquaculture the rainbow trout is the most affected species. The majority of these outbreaks were mainly based on VHS viruses (VHSV) of the Ia–subgenotype. Within the project "Molecular tracing of viral pathogens in aquaculture" (MOLTRAQ) founded by the EC (EMIDA-ERA Net: 2811ERA174), VHSV isolates were collected and characterized genetically on the basis of their glycoprotein gene G. This study includes a phylogenetic network analysis of more than 600 VHSV isolates of the genotype I. The dataset comprises the complete glycoprotein gene sequence from nearly 400 new VHSV samples originated from Denmark, France, Germany, Italy, Netherlands and Switzerland between 1971 and 2014 as well as approximately 200 published VHSV samples. Furthermore, the network was analysed for a phylogeographic and –temporal structure. The results reflect a particularly high genetic diversity of the sub-genogroup Ia. The data clearly indicate population dynamics like demographic expansion events, but also population declines (probably due to eradication measures) of some Ia subpopulations.
In summary it can be stated that the presented study provides a new insight into the evolutionary history of the Ia–subgenotype, and therefore into the epidemiological past of the European VHS disease. A comprehensive dataset including information from reported VHS outbreaks within Europe will improve the reconstruction of epidemiological events.

OSTREID HERPESVIRUS-1 mVAR: EPIDEMIOLOGICAL APPROACHES IN OUTBREAK INVESTIGATION, HYPOTHESIS PROPOSITION AND INTERVENTION STUDIES IN FARMED PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) TO IDENTIFY RISK FACTORS FOR DISEASE CONTROL

R. WHITTINGTON*, I. PAUL-PONT, P. HICK, N. DHAND, O. EVANS, A. RUBIO

Faculty of Veterinary Science, The University of Sydney, Camden, Australia

OsHV-1 mVar emerged in Australia and New Zealand in 2010. This virus devastated *C. gigas* culture in the affected areas and it has not possible to restock without risk of further outbreaks. The purpose of this study was to identify risk factors for mortality events to enable modifications to husbandry practices for disease control. This was achieved through: i) a formal outbreak investigation ii) an intervention study in juvenile and adult oysters and iii) an intervention study in spat. Outbreak investigation in the Hawkesbury river in January 2013 during the first outbreak in this estuary revealed: virus present at low levels in sentinel oysters three months prior to the mass mortality event, probable sequential environmental infection sources, an incubation period < 4 days for mass mortality, spread of virus with estuarine hydrodynamics, inefficient oyster-to-oyster transmission, virus present in all cultivation areas despite absence of disease from some, seawater temperatures above 24°C before and during the outbreak, and higher mortality rates in spat and juveniles than in adults. The first intervention study, conducted in the Georges River estuary over summer 2011-2012 and repeated in summer 2012-2013 confirmed that the mortality rate of adult oysters can be reduced by 50% by reducing the probability of exposure. This was achieved by raising the growing height in intertidal cultivation to reduce immersion time. This approach was not successful for juvenile oysters. Observations of statistically significant non-random viral transmission and mortality at small (cm) and large (km) scales in the estuary led to the hypothesis that OsHV-1 was transmitted on particles, much like plankton. The second intervention study in summer 2013-2014 involved 2 mm spat in seven consecutive trials, and revealed that filtration of seawater to 5 mm to remove putative particle vectors completely prevented mortality. Aging seawater for 48 hours had the same effect. These findings enabled hatcheries in Australia and New Zealand to implement/validate OsHV-1 disease prevention strategies. Integrated disease control involving husbandry modifications based on confirmed risk factors as well as improved genetics are required. The greatest challenge is to overcome mortality in juveniles in an economic way.

ANTIGEN UPTAKE IN ZEBRAFISH

L.V.G. JØRGENSEN*, F. MEHRDANA, P.W. KANIA, M.H. LARSEN, D. FREES, K. BUCHMANN

University of Copenhagen, Copenhagen, Denmark

In aquaculture, immersion-vaccines are routinely used in rainbow trout against Yersinia ruckeri (Yr). The vaccines consist of dead bacteria (bacterin) and follwing immersion lasting at least 30 seconds, rainbow trout take up the dead bacteria and 400 degree days later acquire a level of protection. We have used zebrafish as a model organism to study uptake mechanisms and subsequent transport in fish. A gene-modified Yr was developed to constitutively express GFP (green fluorescent protein) and this bacterium was subsequently used for bacterin production. Transparent zebrafish (Tra:nac) were immersed in this bacterin for up to 30 minutes. Samples were taken after 1 min, 15 min, 30 min, 2 h, 12 h and 24 h. At each sample point two fish were used for live imaging using a stereomicroscope with UV filters. Three fish at each sample point were also sampled for immunohistochemistry (IHC). Results showed that bacteria initially were visible in scale pockets, sometimes on the skin, in the esophagus, in the intestine and in a few instances on the fins. However, within two hours Yr-antigens were visible in the spleen and within 24 hours also in liver and kidney. Bacteria were not visible on the gills or in the blood. These results are in alignment with studies conducted on rainbow trout and zebrafish may therefore be a suitable model for rainbow trout. The next step in this investigation will include the cellular mechanisms responsible for antigen transport.

AEROMONAS SALMONICIDA INFECTION IN VACCINATED RAINBOW TROUT: INFLUENCE OF CHALLENGE METHODS AND ENVIRONMENTAL FACTORS ON CHALLENGE SUCCESS

J.K. CHETTRI[1]*, J. SKOV[1], R.M. JAAFAR[1], B. KROSSØY[2], P.W. KANIA[1], I. DALSGAARD[3], K. BUCHMANN[1]

[1]*University of Copenhagen, Frederiksberg C, Denmark*
[2]*Vaxxinova Norway AS, Bergen, Norway*
[3]*Technical University of Denmark, Frederiksberg C, Denmark*

In vivo testing of any candidate vaccine is influenced by the choice of challenge method and the external environmental conditions. In the present study, a comparative challenge study was performed to evaluate the efficacy of different vaccines against the bacterial pathogen *Aeromonas salmonicida* causing furunculosis. Rainbow trout (*Oncorhynchus mykiss*) were vaccinated with two trivalent adjuvanted experimental vaccines containing formalin-killed *A. salmonicida*, *Vibrio anguillarum* O1 and O2a and a commercial corresponding vaccine (Alpha Ject 3000). Fish were challenged by i.p. injection or cohabitation at both freshwater and saltwater conditions at different time points post vaccination. The cohabitation challenge method represents a more natural mode of infection and provided a better differentiation of vaccine groups compared to i.p. injection of live bacteria. However, cohabitation challenge in saltwater condition produced less mortality compared to freshwater, probably due to the growth inhibition of *A. salmonicida* in saline condition, which was also verified by *in vitro* assay. One of the experimental vaccines emulsified in water in oil adjuvant showed a protection comparable to that of the commercial vaccine with lower side effects as observed by the Speilberg scoring system. Gene expression analysis did not show a clear trend for Th1 or Th2 response in the vaccinated fish. Exposure of fish to saltwater increased the IgT production. Overall, the immune response in vaccinated fish, the side effects due to oil adjuvants, and the importance of choice of challenge methods used will be discussed.

Chettri, J.K., Skov, J., Jaafar, R.M., Krossøy, B., Kania, P.W., Dalsgaard, I., Buchmann, K., 2015. Comparative evaluation of infection methods and environmental factors on challenge success: Aeromonas salmonicida infection in vaccinated rainbow trout. Fish & Shellfish Immunology. 44, 485-495.

INNATE AND ADAPTIVE IMMUNE RESPONSES IN LUMPFISH (*CYCLOPTERUS LUMPUS* L.)

G.T. HAUGLAND[1]*, A. RØNNESETH[1], N. VESTVIK[2], D.B. GHEBRETNSAE[1], H.I. WERGELAND[1]

[1]*University of Bergen, Bergen, Norway*
[2]*Pharmaq, Oslo, Norway*

In the present study, we have characterized the leukocytes and studied the innate and adaptive immune responses in lumpfish (*Cyclopterus lumpus* L.). Leukocytes were isolated from head kidney, peripheral blood and spleen and different subtypes of leukocytes were determined based on morphology and cytochemical staining. IgM^+ B cells were sorted by use of a polyclonal rabbit anti-lumpfish IgM antibody. The innate immune responses, measured by phagocytic and respiratory burst activity, were shown to be very efficient. Different subsets of leukocytes, including IgM^+ B cells had phagocytic activity. The IgM^+ B cells had high phagocytic ability and were the predominant phagocytes in blood with higher capacity than IgM^+ B cells in HKL. Interestingly, the most potent phagocytes were, in addition to monocytes, some small agranular uncharacterized IgM^- cells. Further, the ability to produce specific antibodies towards several bacteria upon immunization verified adaptive immunity in the species.
The high proportion of phagocytic IgM^+ B cells and their phagocytic ability indicate a significant role of phagocytic B cells in lumpfish innate immunity. The present analyses also give strong indications that vaccination and immunostimulation of farmed lumpfish can be used to prevent disease and mortalities caused by pathogenic organisms.

TEMPERATURE DEPENDENT IMMUNE RESPONSE IN ATLANTIC LUMPFISH (*CYCLOPTERUS LUMPUS* L.)

T. ERKINHARJU[1]*, D.H. KNAPPSKOG[2], Ø.B. VÅGNES[2], E. ISDAL[2], R.A. DALMO[1], T. SETERNES[1]

[1]*Norwegian College of Fishery Science, University of Tromsø, Tromsø, Norway*
[2]*Vaxxinova Norway AS, Bergen, Norway*

The use of lumpfish as cleaner fish to prey on salmon lice in aquaculture has increased rapidly in the last years, and bacterial diseases are a major cause of mortality after sea transfer. More basic knowledge of its immune system is needed in order to develop efficacious vaccines. The water temperature levels along the Norwegian coastline changes on a seasonal basis, and how this affect the immune response of lumpfish is not known. We have studied the effects of temperature after vaccination.

We vaccinated 360 lumpfish with either 50 µl of an oil-based injection vaccine, containing inactivated *Aeromonas salmonicida* and *Vibrio salmonicida* antigens, or PBS. Injected fish were divided into 6 groups (n=60) with three different temperature regimes at 5ºC, 10ºC and 15ºC, and were weighed and sampled every three weeks after vaccination until day 126. Blood was withdrawn to prepare serum samples for ELISA, whereas head kidney samples were stored on RNAlater, other organs were fixed using 4% buffered formalin. Vaccine side effects were evaluated by a modification of the Speilberg scoring system on 42 and 63 days post vaccination.

All temperature groups are showing an increase in average weight values. Both vaccinated and control groups at 5ºC and 15ºC demonstrated the same level of weight gain with no clear differences. The control group at 10ºC demonstrates a larger average weight gain than the vaccinated group, which is at the same level as the 15ºC groups on day 84. All the fish in the different groups scored low when evaluating the degree of adhesion of internal organs; 5ºC vaccinated groups and 15ºC vaccinated groups scored 1.45 and 1.8 on day 42, respectively, while 10ºC vaccinated groups scored 1.45 on day 63. None of the vaccinated groups displayed any melanisation. Average score on vaccine residues for the 5ºC and the 15ºC vaccinated groups on day 42 were 1.9 and 1.85, respectively, and 1.5 for the 10ºC on day 63. The control groups scored 0 in all categories. In conclusion there were no significant weight differences, and the side effects were low. Results from the ELISA analysis will be presented.

HEALTHY FISH IN AQUACULTURE: IS *SAA* AN APPROPRIATE MARKER FOR DESCRIPTION OF HEALTH IN MARAENA WHITEFISH?

M. NIPKOW[1]*, A. REBL[1], T. KORYTÁŘ[2], T. GOLDAMMER[1]

[1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany*
[2]*Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Riems, Germany*

Initial breeding attempts of maraena whitefish revealed the fish's sensitivity to infections, especially to furunculosis, caused by the ubiquitous water pathogen *Aeromonas salmonicida*. Since biotic stressors, like pathogens, lead frequently to heavy losses in aquaculture industry, it is our aim to establish a robust and highly productive whitefish breeding strain for local aquaculture that is characterized by low stress susceptibility and efficient pathogen defense. The acute phase response characterizes the early defense against pathogens. One of the key factors of the acute phase response is Serum Amyloid A (SAA). Previous microarray based transcriptome studies of our group in different salmonids identified SAA as a potential marker that indicates fast and effective immune response. In order to characterize SAA expression levels in maraena whitefish, we injected a pathogenic dosage of *Aeromonas salmonicida* into the peritoneum of selected fish in an infection experiment. At 24, 48 and 72 hours post infection we sampled tissue from spleen, kidney, head kidney, and gills and isolated peritoneal cells to gain RNA, which was subsequently converted to cDNA. cDNA was then applied to measure transcript levels by Fluidigm® Real-Time PCR on the BioMark™System. SAA expression levels were elevated compared to non-infected control group in gill, liver and spleen tissue of infected fish at each time point and in peritoneal cells, 72 hours post infection.
In another approach, we are establishing a head kidney primary cell culture for maraena whitefish as an infection model. Hence, we will get a more detailed insight into SAA function and its regulation on the cellular level by direct stimulation of the primary cells with specific pathogen-associated molecular pattern (PAMPs) molecules of Gram-positive and Gram-negative bacteria at controlled levels and time points.

This project is funded by the European Fisheries Fund (EFF) and the Ministry of Agriculture, the Environment and Consumer Protection Mecklenburg-Western Pomerania, Germany (Pilot project VI-560/730-32614).

AGE-DEPENDENT IMMUNITY TO SALMON LICE, *LEPEOPHTHEIRUS SALMONIS*, IN PINK SALMON, *ONCORHYNCHUS GORBUSCHA*

S.R.M. JONES[1,2]*, L.M. BRADEN[2], B.F. KOOP[2]

[1]*Pacific Biological Station, Nanaimo, Canada*
[2]*Department of Biology, University of Victoria, Victoria, Canada*

Pink salmon (*Oncorhynchus gorbuscha*) are semelparous anadromous salmon that migrate to the ocean at mean weights less than 0.5 g. This behaviour exposes the fish to marine pathogens suggesting the early development of defense functions has a selective advantage. Supporting this, rejection of the salmon louse, *Lepeophtheirus salmonis* in juveniles larger than 0.7 g is associated with rapid and robust innate defense responses in the skin. In contrast, adult pink salmon captured at sea or shortly before spawning carry large numbers of the parasite. The purpose of this research was to test the hypothesis that maturing pink salmon have a reduced capacity to control the infection. Pink salmon from a single laboratory population were exposed to adult female *L. salmonis* at ages 7-months (119.3 g) and 19-months (254.1 g). Parasite attachment sites were screened for the expression of genes associated with defense functions and by immunohistochemistry for immune cell markers. The older fish had fully developed gonads, resorbed scales and secondary sexual characteristics. The quantity of mRNA encoding *major histocompatibility factor II*, *C-reactive protein*, *interleukin-1β*, *interleukin-8* and *cyclooxygenase-2* was reduced in mature but not juvenile pink salmon. Similarly, attachment-site skin of the juvenile salmon was highly populated with MHIIβ$^+$ and Il-1β$^+$cells that were either absent, or at reduced levels at similar sites in mature salmon. In addition, mucocyte density was relatively low in the skin of mature salmon, irrespective of louse infection. In juveniles, the higher mucocyte density decreased following louse attachment. We conclude that genetic and histological responses in skin in mature pink salmon are depressed and we speculate that salmonid defenses against *L. salmonis* are modulated by sexual development.

DETERMINING THE PRESENCE AND ROLE OF AN ACQUIRED IMMUNE RESPONSE IN *SALMO SALAR* TO LARVAL SEA LICE INFECTIONS: A MUCOSAL APPROACH

S. MONAGHAN[1]*, H.-Y. PANG [1,2], C. MCNAIR[1], S. HAMILTON[3], A. ADAMS[1], D. KNOX[3], J. IRELAND[1], N. JAYASURIYA[1], H. MCDONALD[1], R. RICHARDS[1], P. SMITH[4], W. ROY[5], D. BASSETT[5], A. PRESTON[5], S. BOYD[5], F. GROVES[5], C. MATTHEW[5], T. KANELLOS[6], J. BRON[1]

[1]*Institute of Aquaculture, University of Stirling, Stirling, U.K.*
[2]*South China Sea Institute of Oceanology, Guangzhou, China*
[3]*Moredun Research Institute, Edinburgh, UK*
[4]*Tethys Aquaculture, Saffron Walden, Essex, UK*
[5]*Marine Environmental Research Laboratory, University of Stirling, Machrihanish, UK*
[6]*Zoetis International Services, Paris, France*

The immune response of Atlantic salmon to *Lepeophtheirus salmonis* can vary depending on parasite life stage. This is largely influenced by parasite immuno-modulatory processes inhibiting the host pro-inflammatory response *e.g.* via IL-1β expression, which prevents larval success in other more resistant salmonid hosts *e.g.* coho or pink salmon, and generally occurs during the first 21 days post infection (dpi) in the absence of established acquired immunity. Secondary challenges appear not to elicit a decrease in parasite numbers relative to an initial challenge, however this has predominantly only been examined at the group level, whereas the large variation of lice infecting individual fish suggests that individuals within each group may be able to reduce louse burden relative to their initial infection. The current study was therefore carried out with re-infections of PIT-tagged Atlantic salmon applied after periods of recovery from previous experimental infection episodes. Two lice counts were conducted at the larval stage (chalimus) and 2 at the motile louse stage following moulting (pre-adults and adults). Although no reduced larval numbers were observed following a second challenge, an interesting variation in louse numbers occurred on individual fish where fish harbouring initially high numbers of chalimus carried relatively fewer lice after moulting to motile stages, whereas fish harbouring initially low numbers of chalimus appeared to gain lice post-moult. Individual fish with apparently reduced louse numbers were compared to those with relatively increased lice by determining the ratio of initial larval infections per fish compared to larval louse numbers following the second challenge. As skin epithelium and mucus make up a large proportion of the louse diet, secreted antigens at this site and the intake of mucosal antibodies may be exploited for vaccination. Therefore, mucus samples were collected and analysed by ELISA, SDS-PAGE and western blot for the presence of salmon immunoglobulins (IgM and IgT) against sea louse extract fractions and sea louse-specific recombinant proteins. In order to elucidate the role of salmon skin mucosal immunity in possible naturally acquired protection against larval sea lice immune transcripts (e.g. IgM, IgT, MHCII, CD4) were also analysed from dorsal fin biopsies and spleen by qPCR.

TRANSMISSION OF PUFFY SKIN CONDITION IN RAINBOW TROUT *ONCORHYNCHUS MYKISS* (WALBAUM) BY CO-HABITATION CHALLENGE

I. CANO*, D. VERNER-JEFFREYS, R. VAN AERLE, G. RIMMER, R. PALEY, S.W. FEIST

Cefas, Weymouth Laboratory, Weymouth, UK

The transmission of puffy skin (PS) condition to rainbow trout *Oncorhynchus mykiss* (Walbaum) was tested by co-habitation challenge with PS-affected fish collected from the field. Two separate challenges, using PS affected fish (Trojans) sourced from two different sites, were conducted. Both challenges lasted for 49 days.

Diploid and triploid naïve fish were used in the infection trials. Development of PS symptoms was observed in both group of naïve fish, in overall 66 % of the fish sampled during the challenge showed PS symptoms of different severity. PS clinically presented first as white oval patches in one or both flanks in the skin after just 15-21 days post challenge (dpc). The extent of the patches ranged between 10 to 90% of the body surface, depending of the severity of the lesion. Severity and number of the fish affected increased during the challenge. Macroscopically, dermal hyperplasia and multifocal petechial haemorrhaging were observed at the end of the trials. Abnormal fish behaviour consisting of "flashing" and excessive mucus production was noted from 15 dpc to the end of the challenge. Fish with severe PS lesions also displayed inappetence and associated emaciation.

Unidentified cells containing rod-like cytoplasmic inclusions were observed in 41% of the fresh skin scrapes analysed from the second trial. *Ichthyobodo necator* was also identified at low levels in in 10% of the skin scrapes analysed. Histologically epidermal oedema was observed in 31% of the naive fish showing gross pathology, with additional 12% displaying epidermal hyperplasia, mostly observed at the end of the challenge. Other concomitant features of the PS lesion developed in the naive fish were epithelial erosion and sloughing, and occasionally mild or focal inflammation.

The parasites *Ichthyophthirius multifiliis* and *I. necator* were observed in a small proportion in the skin of naïve challenged fish and in Trojans but not in control fish. No consistent pathology of internal organs was observed. The results of analysis of metagenome sequence data, obtained by Miseq sequencing of DNA, RNA and rRNA depleted RNA extracted from PS samples, were inconclusive. In summary we have showed that PS is a transmissible condition. However, the aetiology remains elusive.

INFECTION WITH PISCINE REOVIRUS (PRV) FAILS TO CAUSE HEART AND SKELETAL MUSCLE INFLAMMATION (HSMI) IN ATLANTIC (*SALMO SALAR*) AND SOCKEYE SALMON (*ONCORHYNCHUS NERKA*)

K.A. GARVER[1], J.C. BRADSHAW[1], G.D. MARTY[2], H.N. SNYMAN[2], M.P. POLINSKI[1] J. RICHARD[1], D.B. MORRISON[3], S.C. JOHNSON[1]*

[1]*Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo, Canada*
[2]*Animal Health Centre, Ministry of Agriculture, Abbotsford, Canada*
[3]*Marine Harvest Canada, Campbell River, Canada*

Piscine Reovirus (PRV) is a double stranded RNA virus that in Norway is associated with the disease Heart and Skeletal Muscle Inflammation (HSMI). PRV has been identified in wild and farmed salmonids from the West Coast of North America, however examination of hundreds of wild and farmed salmon from the West Coast of North America has to date found no HSMI. We used injection and cohabitation challenge trials to determine (*i*) the infectivity of PRV derived from farmed Atlantic salmon in British Columbia and (*ii*) whether PRV could cause HSMI or other disease in naïve Atlantic and Sockeye Salmon. Following infection, PRV replicated within the hosts and over a 41-week period, PRV persisted in both species without any histopathological signs of HSMI or any other disease. Atlantic salmon were slightly more susceptible to infection than Sockeye Salmon when challenged by co-habitation. Interestingly, based on expression patterns of key genes that are often involved in response to virus, both Atlantic and Sockeye Salmon had a very limited immune response to PRV infection in head kidney tissue and blood, even when challenged by ip injection. Possible reasons why challenge with PRV from BC failed to cause HSMI in our challenge studies are discussed.

SALMON GILL POXVIRUS IS ACCOCIATED WITH ACUTE, HIGH MORTALITY IN ATLANTIC SALMON

M.C. GJESSING*, T. TENGS, M. ALARCON, K.I. LIE, S. YLVING, B. SAURE, L.TRAN, O.B. DALE

Norwegian Veterinary Institute, Oslo, Norway

Poxviruses are large DNA-viruses causing disease in both vertebrates and insects. In fish, the largest vertebrate group, poxvirus like particles have only been detected in diseased farmed koi carp (*Cyprinius carpio* L), ayu (*Plecoglossus altivelis* Temminck & Schlegel) and Atlantic salmon (*Salmo salar* L). Poxvirus infection in salmon was first suspected in the 1990's as electron microscopy showed abnormal gill epithelial cells with poxvirus-like structures from gills of acutely diseased fish. From such gills, we have now characterized the first fish poxvirus genome.

In the gills, the virus was detected in apoptotic epithelial cells from fish in different stages of disease, and the virus was not detected in the control fish. PCR on suspected archival material confirmed the presence of salmon gill poxvirus in cases with gill apoptosis from 1995. We conclude that salmon gill poxvirus is associated with a serious, emerging disease in farmed salmon.

MASS MORTALITIES IN BALTIC SEA EELPOUT (ZOARCES VIVIPAROUS) CAUSED BY A NEW RHABDOVIRUS

M. ARESKOG[1]*, E. BLOMKVIST[1], A. ALFJORDEN[1], M. HAKHVERDYAN[1], T.S. BOUTRUP[2], H. AHOLA[1], F. LJUNGHAGER[3], Å. HAGSTRÖM[1], N.J. OLESEN[2], M. JUREMALM[1], M. LEIJON[1], J.-F. VALARCHER[1], C. AXÉN[1]

[1]*National Veterinary Institute, Uppsala, Sweden*
[2]*Technical University of Denmark, Copenhagen, Denmark*
[3]*Swedish Agency for Marine and Water Management, Gothenburg, Sweden*

In 2014 mass mortalities in eelpout (*Zoarces viviparous*) were reported along the Swedish south-east coast from January to October. Diseased fish were lethargic, lying upright, on the side or upside down, did not move until touched and did not flee far. Movements were uncoordinated, wobbly or circular. Some fish showed breathing difficulties. Necropsy of seven eelpouts did not identify significant lesions. Liver appearance varied whereas gills and spleens were bright red. Microscopically, the brain was the only organ where specific viral lesions was seen. There was cortical histiocytic cell infiltration, single cell neuronal degeneration and perivasculitis. Otherwise, there was generally stasis in investigated organs and a significant amount of intercellular debris was noted in various organs. Some stasis was present in the secondary gill lamellae and there was hypertrophy of the epithelial lamellar cells. The liver parenchyma was hyperplastic with apparent loss of structure, fragmented or duplicate nucleoli and double nuclei. Single cell necrosis and oedema was present in the pancreas. Focal oedema was identified in the kidney interstitium, but there were no significant glomerular changes.
A virus inducing CPE was isolated by cell culture, but ELISAs for VHSV, IHNV, IPNV and SVCV were negative. Chloroform inactivation indicated that it was a rhabdovirus. However, further rhabdovirus testing (hirame rhabdovirus, perch rhabdovirus, *snakehead rhabdovirus, pike fry rhabdovirus, rhabdovirus anguillae and an un-characterized sculpin rhabdovirus*) by IFAT gave negative results. TEM identified a typical bullet-shaped rhabdovirus appr. 140 x 80 nm in size. By deep sequencing of tissue suspension and cell culture supernatant the whole genome was identified, and it had a 59.5% overall match to the closest relative, Siniperca Chuatsi rhabdovirus. Thus, this can be considered a new rhabdovirus, eelpout rhabdovirus (ERV). Symptoms and microscopic lesions in the brain indicate that ERV is neurotropic. A PCR based on the ERV L-gene identified viral RNA in kidney, spleen and heart as well as in the brain (N=5 fish), although in some fish the signal was lower or lacking in the other organs. PCR of organs from healthy eelpout (N=40) was negative, indicating that the virus is associated with the disease and mortalities.

CARP EDEMA VIRUS AND KOI SLEEPY DISEASE IN EUROPE - AN EMERGING ISSUE FROM AN OLD INFECTIOUS DISEASE

B. GORGOGLIONE*, E. LEWISCH, O. SCHACHNER, M. EL-MATBOULI

Clinical Division of Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, Vienna, Austria

Following new outbreaks in several European countries and North America, the Pox-like virus causing Koi Sleepy Disease (KSD), known as Carp Edema Virus (CEV), is raising the attention of fish pathologists and institutions. Although initially reported from juvenile colour carp in Japan in the 1970s, the infection was only detected in Europe in 2008-2009 (in Germany and the UK). In Austria, occurrence of KSD was demonstrated for the first time during spring 2014 in two unrelated cases of common carp and koi carp from earthen ponds. Assuming that the disease has been present for some time, retrospective investigations analysing archived material previously screened for Koi Herpes Virus (KHV) and Spring Viraemia of Carp (SVC) were carried out. Available molecular diagnostic protocols were used and virus propagation on several cell lines attempted. CEV detection from koi carp was tracked back from 2001. Several positive cases were found, especially from 2010 and 2012 (5/13 and 6/28 respectively) from both koi and common carp. Phylogenetic analysis suggested the presence of several virus lineages with a potential divergent region between carp and koi isolates, possibly influencing the current diagnostic test sensitivity. Although only recently attracting attention, CEV segment amplification from old material clearly indicates that the infection is present in Europe since much longer time. For 2015 the first Austrian case arrived in February from koi kept in a garden pond. Co-habited healthy carp showed a transitional viremia, also associated with a sleepy behaviour, limitedly to the fist week post infection. CEV/KSD prevalence is clearly underestimated, above all when considering the low pathogenicity for adult carp and the common camouflaging by other concomitant infections, also including heavy ectoparasitosis. Further investigation is needed to find the susceptible cell line for cultivation of the virus, track back the viral spreading through live stock commercial movements and to precisely assess the current infection risk for ornamental, farmed and wild carp populations in European countries.

MORITELLA VISCOSA IN ICELANDIC LUMPFISH

T. EINARSDOTTIR[1]*, E. BENEDIKTSDOTTIR[2], J. RAU[3], S. HJARTARDOTTIR[1]

[1]*Institute for Experimental Pathology at Keldur, Reykjavik, Iceland*
[2]*University of Iceland, Reykjavik, Iceland*
[3]*The Investigative Office for Food Control and Animal Health, Stuttgart, Germany*

Winter ulcer disease, caused by the psychrophilic bacterium *Moritella viscosa*, primarily affects salmonids, but has also been isolated from other fish species, such as halibut and lumpfish.
In this study, three *M. viscosa* strains were analyzed: (i) a strain isolated 15 years ago from an asymptomatic wild lumpfish in Iceland. (ii) a strain recently isolated from skin ulcers and kidney from captive lumpfish. (iii) a strain isolated from diseased salmon. Experimental infection of salmonids has shown that the virulence of the older lumpfish isolate was moderate. The current isolate of *M. viscosa*, however, appears to be highly virulent in lumpfish.
The aim of this study was to compare the three strains of *M. viscosa*. The 16S rRNA gene and gyrase B genes of all bacterial strains were sequenced and compared. The results indicate that while there is some genetic variability in the lumpfish *M. viscosa* strains, they are more similar to each other than to the salmon strain. This indicates that there is a relatively high level of conservation of the lumpfish *M. viscosa* strains, despite being isolated 15 years apart.
The recently isolated *M. viscosa* came from fish that were kept in captivity. These conditions are likely to be stressful for the fish, and the infectious load may be higher because of the increased density of fish than is expected under natural conditions in the wild.
The bacteria will be subject to mass spectrometry and FT-IR analysis to compare their protein composition and cellular component profiles, respectively. Further research will also focus on experimental infections with the new lumpfish strain to examine its virulence in salmonids and whether the new strain´s virulence is attributable to strain differences or factors such as stressful farming environment.

EARLY MORTALITY SYNDROME (EMS) AS NEW EMERGING THREAT IN SHRIMP INDUSTRY

M.E.J. ZORRIEHZAHRA[1, 2]*, R. BANAEDERAKHSHAN[1]

[1]*Iranian Fisheries Research Organization (IFRO), Tehran, I.R. Iran*
[2]*Iranian Coldwater Fishes Scientific Society (ICFSS), Tehran, I.R. Iran*

So far, emerging and re-emerging diseases should be considered as important events in modern Aquaculture. Early Mortality Syndrome (EMS) also named Acute Hepatopancreatic Necrosis Disease or AHPND should be considered as a new emerging shrimp disease that has been attacked to shrimp farms in Southeast Asia. It was detected in shrimp farms in southern China as first record in 2009 and then in Hainan Island in 2010 and afterward in Viet Nam and Malaysia in 2011 and consequently in the eastern part of Thailand since 2012 and widely spread to other culture areas of Thailand. Its annual losses are estimated more than USD1 billion. Outbreaks of EMS naturally occur in the first 30 days after stocking a freshly arranged shrimp pond, and rate of mortality can pass beyond 70%. The scientists recently found that (EMS/AHPND) could be initiated by a bacterial agent that termed *V. parahaemolyticus* is transferred through oral and then localizes the shrimp gastrointestinal tract and create a poison that causes tissue devastation and invalidism of the shrimp digestive system known as the hepatopancreas. So far, no official report was recorded in human cells. Therefore, it is unlikely that the specific strain of *V. parahaemolyticus* will pose any risk when consumed by human beings. It causes some clinical signs which include pale discoloration and atrophy (size reduction) of hepatopancreas, which appears granular when pressed between the fingers, with occasional black streaks. Other clinical signs include pale and empty stomach and gut, reduced growth, movable shell and black discoloration. Also, lethargy, swimming sluggishly along the dikes, spiral swimming and reduced flourish and feeding are observed in EMS/AHPND. More investigation are needed and should be continue on the development of rapid diagnostic tests for prompt detection of the EMS/ AHPND pathogen that will facilitate developed management of hatcheries and ponds, and could be lead to a long-term elucidation for the disease aspects. So far, no official report about EMS/ AHPND outbreak was recorded in Iran.

NEW ADVANCE ON UDERSTANDING RESISTANCE TO OsHV-1 INFECTION IN *CRASSOSTREA GIGAS*

B. MORGA[1], I. ARZUL[1]*, N. FAURY[1], C. KLOPP[2], F. ESCUDIE[2], T. RENAULT[3]

[1]Ifremer (I*nstitut Français de Recherche pour l'Exploitation de la Mer) Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France*
[2]*INRA (Institut National de la Recherche en Agronomie), Sigenae, UR875, Auzeville, BP 52627, 31326 Castanet-Tolosan, France*
[3]Ifremer *Département Ressources Biologiques et Environnement, Nantes, France*

Mass mortality outbreaks affecting Pacific oyster (*Crassostrea gigas*) spat in various countries are associated with the detection of a herpesvirus called ostreid herpesvirus type 1 (OsHV-1). Few studies have been performed to understand and follow host/viral gene expression, as it has been carried out in vertebrate herpesviruses. In this work, experimental infection trials of *C. gigas* spat with OsHV-1 were conducted in order to test two bi-parental oyster families presenting differential survival rate after an experimental infection and to analyze host-pathogen interactions using in vivo dual RNAseq analysis on oysters after 26h post infection. The divergent response of one oyster family in terms of mortality confirmed that susceptibility to OsHV-1 infection has significant genetic component. After analysis of the total transcriptome of both infected families, high sequence coverage allowed us to thoroughly explore the OsHV-1 transcriptome in a highly OsHV-1 infection susceptible family and identify the activated molecular pathways in *C. gigas* in a low susceptible family. The identification of several highly induced and defense-related oyster transcripts in the most resistant family supports the crucial role played by the innate immune system against the virus.

This work received financial support from the European project "MOLecular TRacing of viral pathogens in aQuaculture" (MOLTRAQ) and the direction scientifique de l'Ifremer GEne SIlencing and autoPHAGIE (GESIPHAGIE).

WHEN LESS IS MORE: EFFECTS OF REDUCED SALINITY ON OSTREID HERPES VIRUS-1 µVAR INFECTION DEVELOPMENT AND MORTALITY IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS)*

S.A. LYNCH*, A. O' REILLY, S. HUTTON, S.C. CULLOTY

School of Biological, Earth and Environmental Sciences, Aquaculture and Fisheries Development Centre and Environmental Research Centre, University College Cork, Cork, Ireland

Global sustainability of the Pacific oyster *Crassostrea gigas* industry faces many challenges, in particular, mortalities associated with ostreid herpes virus-1 (OsHV-1 µVar) infection. Significant losses at all stages of Pacific oyster production have occurred due to this pathogen and control measures to mitigate the impact of this virus are essential to safeguard the future growth of this commercially important oyster species. One of the aims of the REPOSUS project is to ensure that the Irish Pacific oyster industry reaches its Horizon 2020 targets, by ameliorating the impact of OsHV-1 µVar.

The role of salinity on OsHV-1 µVar development was investigated by conducting a laboratory trial with naïve Pacific oyster spat (4 months old, 1.5-3 mm/0.5-2.5 g) sourced from a hatchery in Ireland. Oysters were held in tanks (100 L) at three different salinities (20‰, 35‰ and 38‰) at 22 ºC for 31 days. The trial consisted of 2 control tanks and 3 experimental tanks per treatment, with each tank holding 30 spat. An initial sample (n=60) of oysters was screened by polymerase chain reaction (PCR) to ensure that the spat were uninfected. Prior to commencing the trial, a viral suspension (final conc. of 9.4E04/oyster) was injected into the adductor muscle of each experimental oyster and UV treated seawater into each control oyster. Mortalities were recorded daily for each tank and at the end of the trial all remaining oysters (n=213/450 (47%)) were removed and screened for OsHV-1 µVar by PCR. Cumulative oyster mortality was minimal in the control and experimental tanks at 20‰ (7% and 4% respectively) while highest cumulative mortality was in the experimental tanks held at 38‰ (93%), followed by the control tank at 38‰ (73%). In the 35‰ treatment, mortality was 69% in the experimental tanks and 65% in the control tanks. Prevalence of OsHV-1 µVar infection was 13% at 20‰, 12% at 35‰ and 0% at 38‰. Mortalities were minimal although prevalence of OsHV-1 µVar infection was highest in the 20‰ treatment and even in the absence of OsHV-1 µVar infection high salinity acted as a stressor inducing significant oyster mortality.

THE FIRST DESCRIPTION OF NEW VIRUSES INFECTING THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*)

A.L. ØKLAND[1]*, A. NYLUND[1], A.C. ØVERGÅRD[2], S. BLINDHEIM[1], K. WATANABE[1], S. GROTMOL[1, 3], C.-E. ARNESEN[4], H. PLARRE[1]

[1]*Department of Biology, University of Bergen, Bergen, Norway*
[2]*SLRC-Sea Lice Research Center, Institute of Marine Research, Bergen, Norway*
[3]*SLRC-Sea Lice Research Center, University of Bergen, Bergen, Norway*
[4]*Firda Sjøfarmer AS, Gulen, Norway*

The salmon louse (*Lepeophtheirus salmonis*), feeding on mucus, skin and blood of the host, poses a major challenge for the salmonid farming industry in the North Atlantic. In addition to the damage caused by the parasite it may also be an important vector for several viruses, bacteria and parasites. However, little is known about pathogens infecting salmon louse. Histological examinations have shown the presence of rhabdovirus-like particles in glandular tissue of the salmon louse. Illumina sequencing total-RNA from salmon lice from different Atlantic salmon (*Salmo salar*) farming sites revealed the nearly complete genome of two new viruses. The genomes were around 11 600 nucleotides long and shared the same genome organization as the rhabdovirus genera vesiculovirus, sprivivirus and perhabdovirus. Phylogenetic analysis of the putative N and L proteins shows closest similarity to the Sigmavirus/Dimarhabdovirus cluster, however, the two viruses seem to form a new genus as there is no close affinity to any of the existing rhabdovirus genera. The presence of the viruses in glandular tissue has been confirmed using *in situ* hybridization. Using real time RT-PCR both viruses have been detected in all developmental stages of the salmon louse. This may indicate vertical transmission. The viruses have not been detected in the salmon lice host, Atlantic salmon, with the exception of the attachment site for the chalimi stages.

MOLECULAR MECHANISMS ASSOCIATED WITH WHITE SPOT SYNDROME VIRUS INFECTION/RESISTANCE IN *CARCINUS MAENAS*

R. VAN AERLE[1]*, B. VERBRUGGEN[2], L.K. BICKLEY[2], K.S. BATEMAN[1], E.M. SANTOS[2], C.R. TYLER[2], G.D. STENTIFORD[1]

[1]*European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK*
[2]*University of Exeter, Exeter, UK*

WSSV is one of the most economically significant pathogens in global aquaculture, with devastating effects on the global shrimp farming industry (estimated c. $1.5bn annually). White Spot Disease (WSD) propagates very rapidly and pathological symptoms are characterised by the appearance of white spots in the epidermis, lethargy, loss of feeding and eventually death (within a few days of exposure). Combating this globally important pathogen, therefore, is a high priority worldwide to promote food security and to protect the health of wild crustacean species in areas affected by WSSV. Recent studies demonstrated that European decapods show widely differential susceptibility to WSSV. Experimental infections of the shore crab (*Carcinus maenas*) with WSSV have found limited disease or mortality, suggesting that *C. maenas* may be naturally resistant to WSSV and potentially offering an opportunity to study how this resistance is mediated. In this study, *C. maenas* were infected with WSSV to investigate temporal changes in both the transcriptomic and miRNA responses up to 28 days post-infection. To facilitate this, a reference transcriptome encompassing approximately 212,000 transcripts (representing 62,000 unique annotated genes) was generated from RNA derived from all major tissues. The transcriptome was mined to identify genes from innate immune system pathways including melanisation, JAK-STAT and Toll signalling. Additionally, a draft genome scaffold (approximately 1Gb in size) was generated for *C. maenas*, that will aid in the characterization of regulatory elements, repeat structures and miRNAs. Bioinformatics analyses identified the endocytic pathway as a potentially important regulator of the response to WSSV. In particular, a number of differentially expressed miRNAs were identified that may target Rab7, a regulator of intracellular vesicle trafficking that has previously been shown to bind to WSSV envelope proteins. Differential expression of Rab7 following virus infection was confirmed via QPCR, further supporting its role in the response to WSSV in this species. Gene expression analysis revealed other pathways that may be important in the response of *C. maenas* to WSSV infection. Identification of key molecular targets responsible for resistance to WSSV in species showing low susceptibility may lead to development of preventative and therapeutic interventions for this disease.

EFFECTS OF PARASITES ON THE CONDITION OF MEDITERRANEAN MUSSEL (*MYTILUS GALLOPROVINCIALIS*) WITH RESPECT TO SOME BIOTIC AND ENVIRONMENTAL FACTORS

A. ÖZER[1]*, E. ÇANKAYA[2], S. GÜNEYDAĞ[1]

[1]*Faculty of Fisheries and Aquatic Sciences, Sinop University, Sinop, Turkey*
[2]*Department of Statistics, Faculty of Science and Letters, Sinop University, Sinop, Turkey*

In the present study, we assessed the effects of parasites on the condition of Mediterranean mussel *Mytilus galloprovinciailis* collected from Sinop coasts of the Black Sea. Sampling was conducted monthly from 3 different localities by means of scuba diving during the period from August 2012 to July 2013. A total of 1740 mussels were collected, transported in local water to the parasitology laboratory and kept alive until necropsy. The mussels were then opened and the internal organs were macroscopically and microscopically examined. Parasite morphology was observed using light microscopy. Identified parasites were *Nematopsis legeri, Peniculistoma mytili, Urastoma cyprinae, Parvatrema duboisi* and *Polydora ciliata.* Infection prevalence (%), mean intensity and mean abundance values of each parasite species were calculated according to season, sampling localities and sex of mussels. Condition index was calculated for infected and non-infected individuals of mussels. For statistical analysis, General Linear Models (GLM) using the condition index as the response variable were constructed for the predictors: season, sex, locality, infection status and their possible interactions. Abundances of the most prevalent parasite species were also compared amongst the three localities and all seasons. Results showed that there was no statistically significant difference between the condition of neither infected and non-infected mussels nor different sampling localities and sexes of mussels when the individual effects of each predictor concerned. On the other hand, the seasonal changes played a key role in affecting the condition of analysed mussels. Besides, there appeared significant interactions between season and locality as well as infection status and locality factors.

IMMUNE RESPONSES IN SEA BASS (*DICENTRARCHUS LABRAX*) AND WHITE GROUPER (*EPINEPHELUS AENEUS*) EXPERIMENTALLY INFECTED WITH NERVOUS NECROSIS VIRUS (NNV)

M. UCKO[1]*, S. RAVID- PERETZ[1, 2], K. TARRAB[1, 2]

[1]*Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel*
[2]*Ben Gurion University, Department of Life Sciences, Eilat Campus, Israel*

Viral Nervous Necrosis (VNN) has been causing severe mortalities worldwide in cultured fish, particularly during larval and grow-out stages. The disease has been inflicting tremendous economical losses in Israeli mariculture and threatening the current domestication of the white grouper (*Epinephelus aeneus*) and other commercially important species. Its etiological agent, Nervous Necrosis Virus (NNV), produces necrosis and vacuolization of nervous tissues. Clinical signs include reduced coordination, loss of balance, erratic swimming, and blindness. None of the experimental vaccines developed to date is commercially available. The aim of our study was to characterize the fish immune response as a base for the development of a vaccine.
European seabass (*Dicentrarchus labrax*) and white grouper (*E. aeneus*) weighing ~50g were infected by intramuscular injection (IM) using cell culture propagated NNV. Samples of spleen, kidney, and brain tissue were collected for histopathological, serological and molecular analyses. RT-qPCR and ELISA were developed in order to analyze antibody titers in the serum. Several features of the innate and the adaptive immune system were quantified periodically 1-200 days post-infection (dpi).
Mx gene expression was up-regulated in the brain and blood within 24 hours. In *D. labrax,* anti-NNV IgM increased significantly in the blood within 5 days and lasting at least 180 days. In *E. aeneus*, anti-NNV activity increased significantly in the blood within 12 days, lasting at least 200 days. NNV was detected in the brain within 24 hours and also in the blood for a short period though only in a high dose infection.
A vaccine was produced by inactivation of the virus. The fish were challenged 32 days post-vaccination. Post-challenge mortalities and abnormal behavior were monitored. Vaccinated challenged fish showed significantly lower infection levels compared to the unvaccinated control group. NNV was not detected in any of the brain tissue of the vaccinated unchallenged fish. A significant increase in specific anti-NNV antibodies activity and higher expression levels of interferons (Mx) starting 15 days post-infection were observed in the fish sera and brain tissue. Our results indicate that a vaccine capable of inducing protective immunity that could lead to the effective control of the disease is feasible.

MOLECULAR DETERMINANTS OF BETANODAVIRUS IMMUNOREACTIVITY

V. PANZARIN[1]*, A. TOFFAN[1], M. ABBADI[1], A. BURATIN[1], M. MANCIN[1], S. BRAAEN[2], C. M. OLSEN[2], E. RIMSTAD[2], G. CATTOLI[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy*
[2]*Norwegian University of Life Sciences, Oslo, Norway*

Viral nervous necrosis caused by betanodaviruses is one of the most devastating diseases for the Mediterranean and Asian aquaculture. Betanodaviruses are divided into four genotypes: RGNNV, SJNNV, BFNNV and TPNNV. Besides these four genotypes, reassortant strains RGNNV/SJNNV and SJNNV/RGNNV have also emerged in the Mediterranean area. The genetic diversity of betanodaviruses seems to reflect their serological reactivity, and the existence of three distinct serogroups (serotypes A and B consisting of SJNNV and TPNNV viruses, respectively and serotype C comprising RGNNV and BFNNV type strains) has been proposed. However, information on fish nodaviruses immunoreactivity is still insufficient, and the study of the genetic traits involved in the recognition of viruses by the host immune system is crucial for a targeted diagnosis as well as for the development of effective vaccines.
Rabbit polyclonal antisera against betanodavirus strains rapresentative of the four known genotypes, two types of reassortants, and the cold-water species belonging to the BFNNV group were produced to increase current knowledge on the serological relationships existing among fish nodaviruses. Seroneutralization assays were perfomed testing each serum against an extended panel of viral strains genetically heterogeneous and laboratory results were statistically analysed. To identify genetic determinants responsible for diverse immunoreactivities observed among betanodaviruses, wild-type and chimaeric capsid proteins between the RGNNV and the SJNNV genotypes were expressed *in vitro* and subjected to immunostaining with hyperimune antisera. Our results confirm that the SJNNV and the RGNNV genotypes are serologically distinguishable. Reassortants RGNNV/SJNNV and SJNNV/RGNNV cluster within serotypes A and C, respectively indicating that the coat protein encoded by RNA2 acts as major immunoreactivity determinant. Differently from what has been reported in the literature, the TPNNV and the BFNNV genotypes, including two European cold-water betanodaviruses, are serologically related and cluster within serotype B. The characterization of wild-type and chimaeric recombinant capsid proteins confirms that the RGNNV and the SJNNV genotypes are serologically distinguishable, and highlighs that the C-terminal portion of the capsid protein is involved in the recognition by polyclonal antibodies. Furthermore, data obtained suggest that residues 217-256 and 257-341 are involved in the antigenic recognition of the RGNNV and the SJNNV genotypes, respectively.

This work was funded by the Italian Ministry of Health (RC IZSVE 05/11).

SUSCEPTIBILITY OF TURBOT (*PSETTA MAXIMA*) TO EXPERIMENTAL INFECTION WITH DIFFERENT BETANODAVIRUS STRAINS

S. SOUTO*, J.G. OLVEIRA, I. BANDÍN

Instituto de Acuicultura, Universidad de Santiago de Compostela, Santiago de Compostela, A Coruña, Spain

Viral nervous necrosis (VNN) is a neurological disease that affects a large range of fish worldwide. This pathology is caused by betanodaviruses. These viruses have been classified into four genotypes of which the striped jack nervous necrosis virus (SJNNV) and the red spotted nervous necrosis virus (RGNNV) are the types circulating in Spain. In addition, some reassortant strains between these two types have been isolated from sole and sea bream.

The aim of this study was to assess the susceptibility of turbot to an isolate originated from Senegelese sole, the reassortant strain Ss160.03 (RGNNV/SJNNV). We also investigated the susceptibility of turbot to three recombinant Ss160.03-derived strains ($rSs160.03_{247}$, $rSs160.03270_{270}$ and $rSs160.03_{247+270}$, Souto et al. 2015), in order to investigate the possible role of the amino acids 247 and 270 in host specificity and virulence. Two type strains from SJNNV and RGNNV genotypes were also included in the study for comparative purposes. Turbot juveniles (2g) were experimentally infected by bath immersion with a virus concentration of 10^5 $TCID_{50}$/ml at 18ºC and 16ºC. A time course experiment was also performed at 18ºC in order to compare the replication in the brain tissues of the Ss160.03 and the recombinant strains.

Moderate mortalities (40%-50%) were recorded at 18ºC for the fish infected with the Ss160.03 and RGNNV strains, whereas low mortalities (16.7%) were observed in the group challenged with the SJNNV strain. Regarding the challenges performed with the recombinants, a slight decrease (around 20%) was observed in the mortality rate caused by $rSs160.03_{247}$ and $rSs160.03_{270}$ when compared with that induced by Ss160.03. However, a substantial decrease (60%) was observed in those fish challenged with the $rSs160.03_{247+270}$. Spiral swimming was observed in some fish for all the experimental groups during the experiment. At 16 ºC no clinical signs were observed and mortalities were low (10-20%). The time course assay indicated that the Ss160.03 strain showed the highest capacity to replicate in brain tissues. Virus was detected from day 5 to 30 pi and reached the highest viral load at day 15 pi with a peak of 10^9 viral copies/g. The mutant $rSs160.03_{270}$ was detected also throughout the experiment (peak of 10^7 viral copies/g). However, the recombinant $rSs160.03_{247}$ and $rSs160.03_{247+270}$ were not detected in the brain until 15 dpi and 20 dpi, respectively.

Our results demonstrate that the reassortant betanodavirus is moderately pathogenic for turbot and indicate that amino acids 247 and 270 play a role in virulence.

Souto S, Mérour E, Biacchesi S, Brémont M, Olveira JG, Bandín I. 2015. In vitro and in vivo characterization of molecular determinants of virulence in reassortant betanodavirus. J Gen Virol (in press). http://dx.doi.org/10.1099/vir.0.000064-0

THE INTERACTION OF *BETANODAVIRUS* WITH THE CLAMS, *TAPES PHILIPPINARUM*

S. CIULLI*, B. BRUNETTI, P. SERRATORE, E. VOLPE

Alma Mater Studiorum, University of Bologna, Bologna, Italy

The integrated multi-trophic aquaculture aims to reduce the ecological problems generated by mariculture combining the breeding of different aquatic species such as fish, shellfish and seaweed. The coexistence of fish and invertebrate filter feeders, that concentrate also viruses, can influence the epidemiology of fish infectious diseases. The physical-chemical features of viruses can strongly affect their ability to survive and to be released from the contaminated shellfish, turn it into a dangerous reservoir of the pathogen or an useful "cleaner system" of the surrounding environment such as in the case of IPNV or ISAV respectively.
Betanodavirus, the etiological agent of the Viral Encephalo-Retinophaty, are worldwide spread in farmed and wild finfish. Furthermore, our previous investigations detected betanodaviruses in *Tapes philippinarum, Crassostrea gigas* and *Mytilus galloprovincialis* originating from Mediterranean Sea and Atlantic Ocean. The presence of Betanodavirus in *M. galloprovincialis* was reported also in Korea.
This study aimed to optimize culture and molecular assays to detect *Betanodavirus* in shellfish tissues and to determine the fate of *Betanodavirus* in *T. philippinarum* experimentally contaminated.
Two bioaccumulation assays were arranged to verify the natural ability of the clams to collect virus from contaminated water. Viral presence in hepatopancreas was monitored during the bioaccumulation assays (24 hours) and after transferring the clams in clean water up to 72 hours. *Betanodavirus* was detected and quantify in hepatopancreas with a semiquantitative RT/nestedPCR and with virus culture and titration in SSN-1 cell line.
The histological investigation, 24 hours after *Betanodavirus*-exposure, has not detected appreciable differences between the exposed and unexposed clams.
Culture assay had a detection limit of $10^{2.7}TCID_{50}$/0,1mL. The *Betanodavirus* was consistently detected in the hepatopancreas by both molecular technique and virus culture until 72 hours post infection. Both molecular and titration assays showed no decrease in the viral RNA amount in hepatopancreas 24, 48 and 72 hours post-exposure, reaching the values of $10^{3,77}$ and $10^{4,6}TCID_{50}$/0,1mL in the two bioaccumulation assays.
The results demonstrated that *Betanodavirus* can accumulate and persist alive in clams up to 72 hours post-exposure. Further studies will be done to determine whether *Betanodavirus* is released into the environment, posing a disease risk for cohabited fish.

The project was carried out thanks to funding from Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy.

STUDIES ON *IN VITRO* REPLICATION AND STABILITY OF CYPRINID HERPESVIRUS 3 (CyHV-3)

A.M. BECKER[1]*, A. MLETZKO[1], A. AMTMANN[1], J. CHRISTIAN[1], H. WEDEKIND[3], M. OBERLE[3], R. BUCHHOLZ[1]

[1]*Friedrich-Alexander University of Erlangen-Nürnberg, Erlangen, Germany*
[2]*Bavarian State Research Institute for Agriculture, Starnberg, Germany*
[3]*Bavarian Office for Health and Food Safety, Erlangen, Germany*

Although a lot of attention has been lately directed to Cyprinid herpesvirus 3 (CyHV-3) there is still little information in peer-reviewed literature about its efficient *in vitro* replication. At the same time, low virus titers are not only affecting detection of the pathogen, but also hamper the generation of reliable data concerning its stability and/or of disinfection investigation or make future vaccine production economically unattractive.

Therefore, the aim of this study was to optimize CyHV-3 strain *KHV- TP 30* (Dr. Peiyu Lee, Taiwan, 2005) replication in cell cultures (CCB-common carp brain cells, Neukirch *et al.* 1999) using bioengineering parameters, such as multiplicity of infection (MOI), time of infection (TOI), and time of harvest (TOH), in order to achieve reproducibly high virus titers. After having completed optimization, virus stability at different conditions was investigated.

Virus replication studies showed that using high MOI values does not improve maximum virus yield, but result in lower TOH, whereas TOI has only negligible influence on TOH and can result in reduced virus yield at maximum cell densities. Moreover, applying selected parameters such as: MOI of 0.01, TOI of 100,000 cells/cm^2 and TOH of 3 d.p.i. leads to reproducible titers as high as 1x10^8 plaque forming units (PFU)/mL. Such efficient replication of the virus enabled further stability studies. As expected, this enveloped virus showed to be very sensitive to pH values below 4.5 and above 12 resulting in complete deactivation. Furthermore, the application of commercially available protease (Neutrase®) for virus inactivation also showed very promising results confirming that destroying the virus envelope results in virus inactivation. Next, various disinfection methods, including protease treatment, were additionally tested on the virus adsorbed on pretreated soil samples confirming the efficiency of such deactivation under these conditions. Finally, further feasibility studies will be necessary, but an estimation of costs for application of standard disinfection procedures in comparison to protease treatment already now suggests that the latter can have a great potential for use in aquacultures.

We kindly thank Dr. habil. Sven Bergmann, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald, Germany for providing the virus isolate KHV- TP 30, Dr. Peiyu Lee, Taiwan, 2005. The project was founded by the Federal Organic Agriculture Program (BÖLN), Germany.

EXPERIMENTAL CO-INFECTIONS OF PISCINE ORTHOREOVIRUS AND SALMONID ALPHA VIRUS IN ATLANTIC SALMON (*SALMO SALAR*)

M. LUND[1]*, M.V. RØSÆG[2, 5], I.B. NYMAN[2], Ø. WESSEL[2], V. ASPEHAUG[3], S.M. JØRGENSEN[4], E. RIMSTAD[2], M.K. DAHLE[1]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*Norwegian University of Life Sciences, Oslo, Norway*
[3]*PatoGen AS, Ålesund, Norway*
[4]*NOFIMA, Ås, Norway;* [5]*Salmar ASA, Kverva, Norway*

Piscine orthoreovirus (PRV) is ubiquitous in farmed Atlantic salmon (*Salmo salar)* in the sea along the Norwegian coast. PRV is associated with heart- and skeletal muscle inflammation (HSMI), which is the most frequent viral disease in Norwegian aquaculture. Pancreas disease (PD) caused by salmonid alphavirus (SAV) subtype 2 and 3 are also widespread and causes great concern in Norway. Co-detection of PRV and SAV in fish with histopathological changes resembling HSMI or PD is not uncommon, and raises the hypothesis that PRV-SAV co-infection may influence disease development.

In this study, Atlantic salmon post smolts were exposed to PRV by cohabitation with PRV shedders and then subsequently to a secondary cohabitation challenge using either SAV2 or SAV3 shedders. The SAV shedders were added either 4 weeks post PRV infection which is before the development of HSMI, or 10 weeks after PRV infection, when HSMI pathology starts to resolve. The challenge was terminated after six weeks of PRV-SAV co-challenge.

Regular sampling was performed throughout the challenge trial. RT-qPCR analysis for PRV and SAV loads and transcript profiles of a selection of immune genes are in progress together with histopathological examination.

Preliminary results do not indicate major effects of SAV-infection on the PRV-kinetics as measured by RT-qPCR. However, when SAV was introduced 4 weeks after PRV, the SAV load did not reach as high levels in the co-infection tanks compared to control tanks, which may indicate that the presence of PRV induces anti-viral protection.

Further analysis of histopathology and immune status will be performed to further investigate potential effects of PRV-SAV co-infection.

SALMONID ALPHAVIRUS SUBTYPES 3 AND 2 DID NOT MULTIPLY IN BALLAN WRASSE

T. TAKSDAL[1]*, M. VIKAN RØSÆG[2], D. PERSSON[3], I.MODAHL[1], O. BRECK[4], D. KNAPPSKOG[5], A.B. OLSEN[1], H. SINDRE[1]

[1]*Norwegian Veterinary Institute*
[2]*SalMar, Norway*
[3]*FoMAS – Fiskehelse og miljø, Norway*
[4]*Marine Harvest Norway AS*
[5]*MSD Animal Health, Norway*

In farmed Atlantic salmon (*Salmo Salar L.)* in Norway, infestation of salmon lice (*Lepeoptheirus salmonis*) is an increasing problem. Along with the growth of the industry, the infection pressure of salmon lice is increasing, both between farmed fish and with regard to wild salmonid fish. To reduce the number of lice, "cleaner fish" as wrasse (*Labrus bergylta*) and lumpfish are added to cages with Atlantic salmon to feed on salmon lice. As the supply of cleaner fish is limited, ballan wrasse is to some extent reused, and may pose a risk for transmission of infectious agents between sites.

Pancreas disease (PD), caused by salmonid alphavirus (SAV), is also an increasing problem in Norwegian aquaculture.The routes of spread of the infection to yet non-infected areas can be difficult to track. In order to evaluate if ballan wrasse can be a vector for SAV, experimental infection was performed. As infectious material we used SAV subtype 2 (SAV2) and SAV subtype 3 (SAV3), the two subtypes circulating in Norwegian aquaculture.

In the first experiment, ballan wrasse was injected with either SAV2 or SAV3. A minimum of 20 fish were thereafter collected at each of six samplings until 41 days after injection. Heart and kidney tissues were examined by real time RT-PCR. No multiplication of SAV was detected, although a few samples tested positive with high Ct values.

In the second experiment, we used two tanks, each separated into two compartments by a metal grid. Atlantic salmon and ballan wrasse were placed at each side of the grid, sharing the same water. Atlantic salmon in the two tanks was injected with SAV2 and SAV3, respectively. Later, histopathological and virological examinations confirmed PD in Atlantic salmon in both tanks. Heart and kidney from 25 ballan wrasse were collected and examined weekly from each tank, from 3 to 6 weeks after onset of the challenge. One sample tested SAV positive with a high Ct value. In conclusion, these results indicate that ballan wrasse is not susceptible to SAV.

DOES HOST PLOIDY AFFECT THE PARASITISM LEVEL?

M. DÁVIDOVÁ[1]*, P. HYRŠL[2], M. FLAJŠHANS[3], A. ŠIMKOVÁ[1]

[1]*Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*
[3]*South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Vodňany, Czech Republic*

Polyploidization, a multiplication of the whole chromosome complement, is a widespread phenomenon in plants, which sporadically occurs among animals, including both wild and farmed fishes. Polyploidy is always associated with changes in cell morphology or physiology and can significantly affect individual fitness. On the model of tench *Tinca tinca* (Cyprinidae), a fish species extensively bred in European aquaculture, we tested possible selective advantage in parasite resistance in triploids over diploids. During four samplings (September, December, March and June) 86 diploids and 74 triploids were collected from a breeding pond in Vodňany (Czech Republic). More than 12,000 individuals of parasitic metazoans belonging to 13 species were recorded. Only four species: the monogenean *Gyrodactylus tincae*, the trematode *Asymphylodora tincae*, the larval cestode *Valipora campylancristrota* and unspecified group of Myxozoa spp. occurred throughout the year in higher abundances. Host sex did not affect either parasite abundance or parasite species richness. In concordance with our prediction, diploids tended to have higher parasite load than triploids throughout the year. Nevertheless, significantly higher overall parasite abundance in diploids compared to triploids was confirmed only in summer. Host ploidy appeared to be associated with the susceptibility or resistance to particular parasite species. Whilst diploid tench showed higher susceptibility to endoparasitic trematode *A. tincae*, triploid tench was significantly more parasitized by endoparasites *V. campylancristrota*. The occurrence of *Gyrodactylus tincae* and Myxozoa spp. was only seasonally dependent. Generally no associations between the total parasite abundance and selected haematological, physiological or immune parameters measured were recorded, except for positive correlation between parasite load and the total erythrocyte count in diploids or the leukocyte count in triploids in the summer.

ECIP (GBP505/12/G112).

DETECTION OF SALMONID ALPHAVIRUS IN EXPERIMENTALLY INFECTED DIPLOID AND TRIPLOID ATLANTIC SALMON FRY

T. HERATH[1]*, A. ASHBY[1, 2], N. JAYASURIYA[1], J. BRON[1], R. RICHARDS[1], A. ADAMS[1], M. WEIDMANN[1], J. TAGGART[1], J. TAYLOR[1], H. MIGAUD[1], K. THOMPSON[1,3]

[1]*University of Stirling, UK*
[2]*Fish Vet Group, Inverness, Scotland, UK*
[3]*Moredun Research Institute, Edinburgh UK*

With increasing interest in the use of triploid salmon in commercial aquaculture, it is important to understand how major economically important pathogens affect triploid stocks. The differences in physiology, behaviour and response to stress in triploid fish compared to diploid fish are well described. It is also known that triploid and diploid fish could be equally or more susceptible to ectoparasites. However, information relating to host pathogen interactions of intra-cellar pathogens such as viruses with triploid fish compared to diploid fish is extremely scarce.
To compare diploid and triploid Atlantic salmon, full sib diploid and triploid fry were experimentally infected with SAV sub-type 1 via three routes of exposure; intraperitoneal injection (IP), bath immersion or cohabitation (co-hab) and untreated control group. Fry were monitored for disease progression and mortality until experiment was terminated on 17 days post infection (dpi). Mortalities commenced in co-hab challenged diploid and triploid fish from 11 dpi. Both diploid and triploid IP challenged groups had similar cumulative mortalities at the end of the experiment (41.1% and 38.9% respectively) and these were significantly higher (P value < 0.01) than in the other challenge routes. A Taqman based quantitative PCR was developed to assess SAV load in the heart, a main target organ of the virus, and also in the liver, which does not display any pathological changes during clinical infections, but had exhibited sever degenerative lesions in the present study. In all three challenged groups, in both heart and liver, the median viral RNA copy numbers in the diploid fish was higher compared to triploid fish, however a significant statistical difference was only noted in the liver of co-hab groups. Diploid fry also had significantly higher levels of pancreatic and myocardial degeneration than triploids
This study showed that both diploid and triploid fry are susceptible to experimental SAV1 infection. The lower virus load seen in the triploid fish compared with diploid fry may possibly related to differences between cell transcription efficiencies of the two groups. However, further investigations would be necessary to confirm whether these findings represent the outcome of an outbreak of SAV under field conditions.

THE EUROPEAN CHUB (*SQUALIUS CEPHALUS*) AS A MODEL ORGANISM FOR STUDYING HOST-PARASITE INTERACTION AT THE MOLECULAR LEVEL

M. SEIFERTOVÁ[1]*, J. JARKOVSKÝ[2], A. ŠIMKOVÁ[1]

[1]*Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Institute of Biostatistics and Analyses, Faculty of Medicine and Faculty of Science, Masaryk University, Brno, Czech Republic*

The European chub (*Squalius cephalus*, Linnaeus, 1758) represents a widely distributed cyprinid fish in lotic waters across Europe. As an omnivorous species and a food generalist, it harbours rich and diverse metazoan parasite fauna. Genetic variability of this species is not influenced by fish stocking due to its generally low economic significance. Therefore it represents a suitable model to investigate the mechanisms of parasite-mediated balancing selection under natural condition in the context of neutral evolution. In vertebrates, the genes of major histocompatibility complex (MHC) encode proteins that recognize and present pathogen-derived peptides to T-cells. Among other factors, the MHC is expected to have evolved in response to variation in parasite community. Thus, the MHC genes provide an excellent opportunity to study host-parasite interaction at the molecular level. The main objectives of this study were to test whether there was an association between MHC class II*B* (*DAB*) genes and metazoan parasite infection in European chub populations across its distribution range in Europe. In addition, the variability of twelve microsatellite loci as neutral genetic markers was analyzed to clarify the potential contribution of neutral evolutionary processes to MHC polymorphism. We found significant differences in the metazoan parasite load among the examined European chub populations and also documented a high degree of polymorphism in functional *DAB* genes. The analysis of twelve microsatellite loci revealed significant phylogeographic population structure. The analyses based on pairwise data revealed that populations with dissimilar MHC allelic profiles were geographically distant populations with significantly different diversity in microsatellites and a dissimilar composition of parasite communities. Further, metazoan parasite load in European chub was influenced by the diversity of *DAB* alleles as well as by the diversity of neutral genetic markers and host condition and immunocompetence. We concluded that current MHC diversity in European chub is an outcome of more complex influences than just parasite-mediated selection. We believe that the main role in MHC class II*B* interpopulation diversity was played by neutral evolutionary processes.

This study was supported by ECIP - Centre of excellence, Czech Science Foundation (Project No. P505/12/G112).

RESOLUTION OF *KUDOA THYRSITES* INFECTION IS ASSOCIATED WITH INFILTRATION OF MHIIβ+ CELLS IN ATLANTIC SALMON, *SALMO SALAR*

L.M. BRADEN[1]*, S.D. CHO[2], M.D. FAST[1], S.R.M. JONES[2]

[1]*Atlantic Veterinary College, Charlottetown, P.E., Canada*
[2]*Pacific Biological Station, Nanaimo, B.C., Canada*

Kudoa thyrsites is a myxozoan parasite of the skeletal muscle in a wide range of fish hosts with a global distribution. In British Columbia Canada, infections in farmed Atlantic salmon (*Salmo salar*) incur significant economic losses due to post-mortem myoliquefaction. Despite obvious commercial importance, little is known about the life-cycle or host-parasite relationship of *K. thyrsites*. Salmon recover from experimental infections and the recovery process is characterized by a gradual loss of plasmodium structure and replacement with fibrous connective tissue. The cellular mechanisms responsible for this process are not known although macrophage-like phagocytes containing mature spores have been observed in chronically infected fish. To address the possibility of protective adaptive immunity in recovered fish, Atlantic salmon were exposed to infective seawater for 500 or 1000 degree-days (DD). The fish were then maintained in UV-sterilized seawater and muscle samples were examined histologically at 2000, 3500 and 4312 DD. Previously exposed fish and unexposed controls were exposed to infective seawater from 4312 to 4812 DD and histological examinations conducted at 6312 DD. Prevalence and severity of *K. thyrsites* declined significantly between 2000 and 4312 DD and there was no statistical difference between the exposure groups. Following re-exposure, the prevalence and severity of infection were significantly lower in previously exposed salmon compared with controls. Significant infiltration of MHIIβ+ cells was detected in the musculature of infected salmon compared to uninfected salmon. The association of these cells with infected myocytes proceeded in 4 stages: initial contact and envelopment of the myocyte, infiltration of the myocyte, envelopment of the plasmodium and complete degradation of the plasmodium and dissemination of spores by positive cells. While not yet identified, the MHIIβ+ cells appear to play an important role in the host response to *K. thyrsites*. This study provides evidence for acquired resistance to reinfection of Atlantic salmon with *K. thyrsites*.

HOST SUSCEPTIBILITY TO INFECTION BY THE SALMON LOUSE, *LEPEOPHTHEIRUS SALMONIS*, INVOLVES REPSONSES BY SALMON AND PARASITE

L.M. BRADEN*[1], B.J.G. SUTHERLAND[1], D.R. MINKLEY[1], B.F. KOOP[1], S.R.M. JONES[1,2]

[1]*University of Victoria, Victoria, B.C., Canada*
[2]*Pacific Biological Station, Nanaimo, B.C., Canada*

Salmon is the largest Canadian mariculture and production is projected to reach 197,000 tonnes and $1.2 billion revenue by 2020. In the midst of this growth, it remains imperative for the industry to minimize negative ecological and environmental impacts. One such impact is the spread of the parasitic salmon louse, *Lepeophtheirus salmonis*. There are species-specific responses to infections with *L. salmonis* that contribute to more resistant (i.e. coho salmon, *Oncorhynchus kisutch*) or susceptible (i.e. sockeye salmon, *O. nerka*; Atlantic salmo, *Salmo salar*) host phenotypes. In the face of diminishing chemotherapeutant efficacy against the louse, understanding the basis for these divergent responses is essential for developing novel control strategies. *L. salmonis* secretes various bioactive compounds (virulence factors) which act to suppress host inflammatory responses and aid in feeding. Thus the objective of this work was to explore the parasite response during infection of *L. salmonis* on resistant and susceptible salmon species. To test the hypothesis that *L. salmonis* exhibits host-specific feeding responses, we applied a 38K oligonucleotide microarray to profile the parasite's transcriptome during feeding after 24 and 48 hrs on Atlantic, coho and sockeye salmon, or after starvation (24 and 48 hrs). Lice fed on all species; however, there were significant differences between feeding responses on Atlantic, coho or sockeye salmon in the number and function of differentially expressed genes. Expression profiles of virulence factors (e.g. trypsins, carboxypeptidase, cathepsins), protein synthesis (e.g. ribosomal proteins, histones) and energy production (eg. cytochrome oxidases) were increased over time during attachment on Atlantic salmon, while on coho or sockeye salmon the response decreased and more closely resembled profiles observed for starved *L. salmonis*. This is the first evidence that *L. salmonis* exhibits genus-specific feeding responses that may contribute to host-susceptibility. Thus we propose that susceptibility to infection with the salmon louse involves responses by the host (e.g., inflammatory response, cellular effectors, wound healing) and the parasite (e.g., exaggerated expression of virulence factors). Identification of salmonid cutaneous factors that contribute to the divergent lice feeding responses among Atlantic and Pacific salmon will be invaluable for development of novel control strategies.

CHARACTERISATION OF CYSTEINE PROTEASES FROM THE SALMON LOUSE, *LEPEOPHTHEIRUS SALMONIS,* AND THEIR POTENTIAL AS VACCINE CANDIDATES

C. MCNAIR[1]*, S. MONAGHAN[1], S. HAMILTON[2], J. IRELAND[1], D. KNOX[2], J. BRON[1]

[1]*University of Stirling, Stirling, UK*
[2]*Moredun Research Institute, Edinburgh, UK*

Sea lice are a major pest of both farmed and wild Atlantic salmon (*Salmo salar*) worldwide. The most important species found in the UK is the salmon louse, *Lepeophtheirus salmonis*. Salmon lice cost the Scottish aquaculture industry an estimated €33 million each year, making them an important concern for salmon farmers. Treatment of lice is usually through the use of chemotherapeutants, however it has become increasingly apparent over the last 15 years that this is unsustainable, and so alternative control methods are needed. One potential alternative method of control is the development of a vaccine to protect fish from salmon louse infection. Anti-parasitic vaccines require the identification of antigens which are essential to the survival of the target species, for example digestive proteases or proteins involved in parasite establishment or reproduction. Cysteine proteases have been characterised as potential vaccine candidates from several other parasite species, including gastrointestinal nematodes and liver fluke, with some success. These enzymes have a variety of roles in parasites, including blood meal digestion, reproduction, and parasite establishment. In this study, three cysteine proteases from the salmon louse were characterised through a variety of experiments, and their potential for use in an anti-louse vaccine evaluated.

Cathepsin B (CB), cathepsin L1 (CL1) and cathepsin L2 (CL2) were amplified by PCR from various stages of *L. salmonis*, from eggs through to adults, to analyse expression patterns through the lifecycle. All three proteases were found to be strongly expressed in adult males and females, and at varying levels in different larval stages. Expression of CB, CL1 and CL2 in the gut was confirmed by RT-PCR of gut-specific RNA isolated using laser capture microdissection (LCM). *In situ* hybridisation was also used to further localise expression. Recombinant versions of CB, CL1 and CL2 were produced in *E. coli*, and will be used for Western Blots and ELISAs to establish whether or not these proteases are recognised by mucus from infected fish. Based on the results of these experiments, these proteins may be used in future small scale vaccine trials to assess their suitability as protective antigens in salmon louse infection.

DIFFERENTIAL INDUCTION OF IMMUNE GENE EXPRESSION IN DIFFERENT ATLANTIC SALMON (*SALMO SALAR L.*) TISSUES AT EARLY STAGES OF LICE INFECTION

A.A.A. GAMIL[1]*, T.C. GUO[1], S. MUTOLOKI[1], S.DALVIN[2], C. EICHNER[3], Ø. EVENSEN[1]

[1]*Sea Lice Research Centre, Norwegian University of Life Sciences, Oslo, Norway*
[2]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*
[3]*Sea Lice Research Centre, University of Bergen, Bergen, Norway*

Sea lice (*Lepeophtheirus salmonis*) infestation is a growing problem for salmonid industry especially with the emergence of strains that resists the available antiparasitic remedies. Atlantic salmon is highly susceptible due to the lack of or poor induction of immune responses compared to other salmonid species like Coho salmon. However, the mechanisms by which the parasite dampens the immune responses in Atlantic salmon are yet to be elucidated. Understanding the interplay between host and parasite is essential to unravel these mechanisms and can therefore help in developing new effective therapeutic strategies that elicit the right immune responses. In this study we have investigated changes in gene expression associated with early stages of lice infection. Atlantic salmon were either infected with sea lice copepodites or left uninfected. At 5 days post infection fish were sacrificed and skin, fin, headkidney and spleen were collected for RNA extraction and subsequently gene expression analysis using real-time PCR. The differential expression of selected inflammatory cytokines including IFNγ, TNFa and IL1B, chemotactic cytokines including CXCL-10 and IL-8 in addition to some other inflammatory markers associated with different cell types was investigated. The findings from this study will be presented and discussed.

TWO PROTOZOAN PARASITES TAKE UP RESIDENCE IN A SAME POPULATION OF FLAT OYSTER, OSTREA EDULIS, IN RADE DE BREST, BRITTANY, FRANCE

I. ARZUL[1]*, L. BAILLON[1], B. CHOLLET[1], C. DUBREUIL[1], C. GARCIA[1], P. HELOURY[1], M. HUSSENOT[2], D. SERPIN[1], S. POUVREAU[3]

[1]*Laboratoire Génétique et Pathologie des Mollusques Marins Ifremer La Tremblade*
[2]*CRC Bretagne Nord*
[3]*Laboratoire des Sciences de l' Environnement Marin (LEMAR), UMR 6539 CNRS/UBO/IRD/Ifremer*

Although the flat oyster, *Ostrea edulis*, is naturally present in Rade de Brest, Brittany (France), density of populations remains very low (< 1 indiv.m^{-2}). The production of this species is locally hampered by several factors: fishing activities, predation (starfish, oyster drill and daurade) but also two constraining diseases, marteiliosis and bonamiosis. These diseases are due to the protozoan parasites *Marteilia refringens* and *Bonamia ostreae*, which are reported in Rade de Brest since 1974 and 1980, respectively.
Eradication of these diseases is not possible and a better understanding of the dynamics of the parasites in the oysters is required to identify their respective impact on the oyster populations and to come out with relevant stock management, conservation or restoration measures.
In this context, we have investigated the presence of these both parasites by PCR in a cohort of oysters from spat to 3 years-old adults. Spat was sampled monthly and oysters older than 9 months were kept in bags and collected every 4 months. Oysters found infected by PCR were selected for further characterization analysis. Histology and *in situ* hybridization allowed estimating the level of infection and describing the distribution and development of the parasite in the tissues. In addition, PCR products were sequenced in order to evaluate the diversity of these parasite species at the oyster population level.
Our results show that both diseases evolve concurrently in the same oyster population but display different dynamics pattern. Marteiliosis seems to occur in spat when environmental conditions are suitable for the transmission of the parasite, which then presents several infection cycles with moderate prevalence. *B. ostreae* was continuously detected in oysters older than 18 months with two peaks of prevalence preceding periods of mortality.
Each of these endemic diseases may deplete host resources, which in turn could affect the development of the other parasite during co-infection. These results highlight the interest of investigating the dynamic of multiple co-infections and within-host competition.

LIFE IN THE SLOW LANE MAY BE A GOOD STRATEGY FOR AN OYSTER'S HEALTH: A SLOWER GROWING NATIVE FLAT OYSTER (*OSTREA EDULIS*) STOCK EXHIBITS REDUCED SUSCEPTIBILITY TO THE PROTISTAN PARASITE (*BONAMIA OSTREAE)*

S.A. LYNCH*, E. BRENNAN, G. FLANNERY, R. MCALLEN, S.C. CULLOTY

School of Biological, Earth and Environmental Sciences, Aquaculture and Fisheries Development Centre and Environmental Research Centre, University College Cork, Cork, Ireland

Several diseases have significantly affected the native flat oyster *Ostrea edulis* industry in Europe; in particular bonamiosis caused by the protistan *Bonamia ostreae*. Extensive research has been conducted to find ways to ameliorate the impact of this pathogen on *O. edulis,* including selective breeding programs for disease resistant stocks.
Several laboratory trials were carried out to determine disease development and immunological response in three Irish *O. edulis* stocks with differing histories of exposure to *B. ostreae*, one short-term (Lough Foyle) with ten years exposure to *B. ostreae*, one long-term (Clew Bay) with seventeen years exposure and one with over thirty years exposure (Rossmore), which is considered to have some resistance to this pathogen. In addition, a naïve Scottish population (Loch Ryan) with no prior exposure to *B. ostreae* was also exposed for comparison. Oysters were held in 500L tanks for 3 weeks and 16 weeks and were infected with *B. ostreae* via natural exposure in the field, direct oyster-to-oyster transmission and the addition of infected homogenised oyster tissue ($1x10^6$ *B. ostreae* cells). Oyster samples were screened using heart imprints, polymerase chain reaction (PCR) and *in-situ* hybridisation (ISH) for prevalence, intensity and progression of infection. Host immune response was assessed using haemolymph cell monolayers and several immunoassays. Lough Foyle and Clew Bay oysters showed an increase in prevalence of infection over each trial period, reaching Class 3 and Class 4 intensities of infection, while Rossmore oysters had a significantly lower prevalence of infection and Class 1 infection. Of significance, Loch Ryan oysters were negative for *B. ostreae* infection in the heart imprints and PCR screening, yet *B. ostreae* DNA was detected in the ISH in 51% (53/104) of the oysters in the 16 week trial. Immune function in the Loch Ryan oysters was similar to that in the "resistant" Rossmore oysters. It is possible that latent infections were being observed in Loch Ryan oysters or that this slow growing stock can become infected but are able to eliminate this pathogen, as additional field trials carried out in UCC in a *B. ostreae*-endemic site have had no success in infecting this stock.

FIRST DETECTION OF *BONAMIA OSTREAE* IN NATIVE FLAT OYSTERS FROM THE LIMFJORD IN DENMARK

L. MADSEN*, H. THOMASSEN

National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark

Limfjorden in Denmark is recognized as a unique production area for European flat oysters, *Ostrea edulis*. In 1980 Bonamia sp. was found in relayed French oysters that had experienced high mortalities, and attempts were made to clean the site by fishing up the whole batch of French oysters. In the 1990's, the native stock of flat oysters in Limfjorden increased and a surveillance program for *B. ostreae* and *Marteilia refringens* was initiated. Limfjorden gained its disease-free status regarding the two parasites in 2004. Regular samplings from three sites twice a year have been done since 2000, and histology have been performed on the oysters. Neither of the parasites had been found, until the autumn sampling in 2014, where *Bonamia*-like cells were seen in sampled oysters. Of the three sites sampled, two were found to be positive. Heart imprints confirmed the histological findings of *Bonamia*, as well as PCR investigations on frozen tissues from the same oysters. The EURL for mollusc diseases in La Tremblade, France (also OIE reference laboratory for bonamiosis) confirmed the findings, both by histology as well as *in situ* hybridisation for *Bonamia*. RFLP and sequencing of the PCR products showed that the *Bonamia* sp. could be classified as being *Bonamia ostreae*. It is the first time that the parasite has been found in native European flat oysters in Denmark. Comparison of results by histology, heart imprints and PCR will be presented, as well as sequencing results.

APOPTOSIS: A DEFENSE MECHANISM USED BY THE FLAT OYSTER, *OSTREA EDULIS*, IN RESPONSE TO THE PROTOZOAN PARASITE *BONAMIA OSTREAE*

O. GERVAIS[1]*, B. CHOLLET[1], T. RENAULT[2], I. ARZUL[1]

[1]*Ifremer, Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France*
[2]*Ifremer, Département Ressources Biologiques et Environnement, Nantes, France*

Apoptosis is a biological process involved in various mechanisms like homeostasis, embryogenesis and defense against stress factors. In molluscs, this mechanism seems to be an important way to fight pathogens by eliminating infected cells. Previous studies reported a modulation of the expression of genes involved in the apoptosis pathway including IAP and Fas ligand in the flat oyster *Ostrea edulis* during an infection with *Bonamia ostreae*. This protozoan infects and multiplies within hemocytes and like other intracellular parasites may develop strategies to modulate apoptosis from the host in order to survive in the target cells.
In this context, we have investigated *in vitro* the apoptotic response of the flat oyster to *Bonamia ostreae* in hemocytes in contact with live and dead parasites. Similarly *in vitro* experiments were performed using hemocytes from the Pacific oyster *Crassostrea gigas*, a species not susceptible to the parasitic disease.
Flow cytometry was used to measure different cellular parameters related to apoptosis including phosphatidyl serine externalisation, intracytoplasmic calcium concentration and mitochondrial membrane potential. Transmission electron microscopy and light microscopy allowed evaluating ultrastructural modifications and DNA fragmentation of infected hemocytes, respectively.
Although apoptosis appeared increased in hemocytes in contact with live parasites whatever the oyster species was, the difference between live and dead parasites was less pronounced in *C. gigas* in comparison with *O. edulis*. Our results suggest that apoptosis is differently activated in the two oyster species. In the flat oyster apoptosis appears as a specific response to live *B. ostreae* whereas in the Pacific oyster this mechanism seems to be a more general response to microorganisms.

PREVALENCE OF THE PROTOZOAN PARASITE (*HAPLOSPORIUM NELSONI*) OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) IN THE DAMARISCOTTA ESTUARY, MAINE, USA

T.J. BOWDEN, J. VIGUE, J. SICOTTE, K. JOHNDROW, N. MESSERMAN

University of Maine, 5735 Hitchner Hall, Orono, ME 04469-5735, USA

Haplosporidium nelsoni is a protozoan parasite that causes a devastating disease in the Eastern oyster, *Crassostrea virginica,* along the East of North America. Until recently, *H. nelsoni* did not reach epizootic levels in Maine. However, in the summer of 2010, *H. nelsoni* was responsible for significant mortalities among cultivated oysters in the Damariscotta River estuary and mortalities have persisted since. This study investigated the prevalence of *H. nelsoni* in one commercial oyster site and several, geographically distinct, natural oyster beds located in the Damariscotta river estuary by using histology and PCR-based assay specific for *H. nelsoni*. In 2011 the local industry changed the oyster strain being grown from one that was cold tolerant but MSX susceptible to one that was MSX resistant. An initial study was conducted in 2012 and a follow-up study completed in 2014. It was suspected that in 2012 the natural beds were still populated with the susceptible strain of oyster but that by 2014 resistant oysters would have become dominant. The prevalence in 2012 was as high as 50% at some sites, while in 2014 the prevalence was substantially lower. This indicates that the change in oyster strain grown commercially also impacted the natural bed populations.

PERFORMANCE CHARACTERISTICS OF POLYMERASE CHAIN REACTION AND HISTOLOGICAL METHODS FOR THE DETECTION OF *HAPLOSPORIDIUM NELSONI* IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*: APPLICATION TO SURVEILLANCE

N. GAGNÉ[1]*, A. VENIOT[1], M. STEPHENSON[1], C. MCCLURE[2]

[1]*Department of Fisheries & Oceans Canada, Gulf Fisheries Center, Moncton, New Brunswick, Canada*
[2]*AquaEpi Research, Charlottetown, Prince Edward Island, Canada*

Fitness for purpose and validation are increasingly becoming a benchmark in the development of test methods for the diagnosis of infectious diseases in aquatic animals. The design of the evaluation and the analysis of data are critical to demonstrate test method performance characteristics and fitness for purpose, as stated in the Office International des Epizooties pathway for test validation. Three test methods for the detection of the oyster parasite *Haplosporidium nelsoni* were selected for the validation study described here: histology, end-point polymerase chain reaction (PCR) and real-time PCR (qPCR). Preliminary work evaluated the analytical sensitivity and specificity of the PCR and qPCR assay in development. The following stage used testing on 100 oysters in 3 different laboratories to assess, repeatability and reproducibility. Repeatability and reproducibility were within 68% to 95%. The final part of the project evaluated diagnostic sensitivity (DSe) and specificity (DSp) using tests on 400 oysters and results from the first 100 oysters tested. In the absence of a 100% gold standard test, latent class modelling methods were explored to characterize the tests, i.e. Bayesian analyses. DSe was above 90% for both PCR methods, and in the 60% range for histology, whereas DSp was above 90% for all methods. Based on the results of this validation, a cycle threshold of 30 for qPCR corresponds to the limit of sensitivity for histology where unreliable detection becomes more frequent, thus providing a threshold helpful in diagnostic settings where both histology and qPCR are used. Both histology and PCR/qPCR have been used in surveillance on the East Coast of Canada since the discovery of MSX in 2002, and have proven useful to monitor the spread of the pathogen, as illustrated by the geo-temporal mapping of PCR and histological detection. The qPCR is also useful to detect the pathogen in various potential vectors.

METAGENOMIC ANALYSES OF BACTERIAL FLORA IN THE POND WATER AND INTESTINE OF TILAPIA IN THAILAND

T. AOKI[1]*, U. WATANABE[2], H. TAKEYAMA[2], W. SUDA[3], S. HATTORI[3], J. HIKIMA[4], M. SAKAI[4], S. UNAJAK[5], M. RAPEEPAT[6], H.C. WANG[7], C.F. LO[7]

[1]*Research Organization for Nano and life Innovation, Waseda University, Tokyo, Japan*
[2]*Faculty of Science and Engineering, Waseda University, Tokyo, Japan*
[3]*Center for Omics and Bioinformatics, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan*
[4]*Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan*
[5]*Department of Biochemistry, Faculty of Science, Kasesart University, Bangkok, Thailand*
[6]*Charoen Pokphand Foods Public Co. Ltd, Bangkok, Thailand*
[7]*College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan*

Bacterial infectious diseases occurring in tilapia farm have made huge loss in Thailand. It is highly important to understand basic information of the microbiome to treat these diseases. In previous studies, culture-dependant analysis to detect the intestinal flora of tilapia has been limited because 99% of bacterium cannot be cultivated. Therefore, culture-independent methods should be required. In this study, comprehensive analyses of the microbial flora from farming pond water and intestinal content of tilapia non-treated and treated with oxytetracycline (OTC) or probiotics in Thailand were conducted by using the high-throughput next generation sequencer (NGS). Genomic DNA was extracted from pond water and the intestinal contents of tilapia, respectively. Subsequently, the V1-2 region of the bacterial 16S rDNA was amplified from the extracted DNA using the universal primer set (27Fmod-338R). The amplicons were analyzed using a 454 GS Junior sequencer. To conduct diversity analysis of the intestinal flora, high-quality reads were clustered with 96% identity using UCLUST to construct operational taxonomic units (OTUs). The nucleotide sequence of each OTU was analyzed by BLAST search, and the species populations of microorganism were identified accordingly. Furthermore, the structural similarity of intestinal flora was visualized by Unifrac analysis. In comparison between the pond water and the non-treated intestinal flora, the non-treated intestinal flora was dominated mainly by *Clostridium*, *Bacteroides*, *Cetobacterium* while the pond water flora was dominated mainly by *Polynucleobacter*, *Mycobacterium*, *Acidovorax*. As observed, *Mycobacterium* was the only genus that was detected from both the samples. In comparison between OTC- or probiotics-treated tilapia, *Firmcutes* was significantly abundant in the OTC-treated tilapia intestine while *Nordella* was significantly abundant in the probiotics-treated groups. A few % of *Ediwardsiella* and *Aeromonas* were detected in both group. The Unifrac clusters of intestinal flora of OTC- and probiotics-treated tilapia were obviously separated. These results suggested that the 16S rDNA-based metagenome analysis was useful for identification of the non-culturable bacterial species. Moreover, further metagenomic analysis of such a intestinal flora could make us understand a role of intestinal flora against fish pathogenic bacteria and a relationship between intestinal flora and mucosal immunity in fish.

BACTERIAL DISEASES AND VACCINATION OF FARMED LUMPFISH (*CYCLOPTERUS LUMPUS* L.)

A. RØNNESETH[1]*, G.T. HAUGLAND[1], E. BRUDAL[2], H.I. WERGELAND[1]

[1]*University of Bergen, Bergen, Norway*
[2]*Pharmaq, Oslo, Norway*

Farmed lumpfish (*Cyclopterus lumpus* L.) is with great success used for biological control of salmon louse (*Lepeoptheirus salmonis*). Along with the rapid increase in the farming of lumpfish in Norway bacterial disease problems has emerged. Most disease outbreaks have been caused by infections with atypical *Aeromonas salmonicida*, *Vibrio anguillarum* or *Pasteurella*. In these studies we have tested the susceptibility of healthy lumpfish when challenged experimentally with bacterial strains isolated after outbreak of disease in lumpfish. Concerning atypical strains of *Aeromonas salmonicida*, there was a clear difference in pathogenicity between isolates expressing the A-layer protein and isolates not expressing of this protein. Based on the established challenge models and characterizations of the different bacterial strains we have developed and tested vaccines towards lumpfish pathogens and the results indicate that vaccination will be successful for this species.

MOST IMPORTANT TILAPIA INFECTIOUS DISEASES (BACTERIAL AND VIRAL) IN HATCHERY PHASE TILL OFFER TO THE MARKET (GROW OUT PHASE)

M.E.J. ZORRIEHZAHRA[1]*, M.R. MEHRABI[1], S.T. DELSHAD[2], M. ADDEL[1]

[1]*Iranian Fisheries Research Organization (IFRO), Tehran, I.R. Iran*
[2]*Shiraz University, School of Veterinary Medicine, Shiraz, I.R. Iran*

Tilapia could be one of important culture fish species and has important role in aquaculture and fisheries economics in the world. It was accepted as culture species in more 100 countries in the world in order to some specificity such as adaptation ability in new environment, rapid reproduction and suitable growth. So far, some important infectious diseases such as bacterial and viral diseases could be considered as one of important basic challenge in aquaculture development. Recent investigation on Tilapia approved that after introduce of Tilapia fry to culture ponds probability of infection to some bacterial and parasites (Monogenes and Protozoan) would be increased after short period. Also, Iridovirus infection could be caused of heavy mortality in early stages of Tilapia culture. Furthermore, *Flavobacterium columnare* infection is a common pathogen in early stages. The most important threats of the mid and last stages of Tilapia culture could be due to some bacterial pathogens such as streptococcal disease that killing big fish from 150 – 300 g onward, as the most important pathogen affecting the Tilapia culture industry. Well-known species of Streptococcus consist of (*S. dysgalacti*), (*S. iniae*) and (*S. agalactiae*) could be produced some clinical signs such as septicemia, meningoencephalitis, loss of appetite, emaciation, lethargy, diffuse bleeding on skin and internal organs, exophthalmia, spiral swimming and heavy mortality in affected Tilapia. Furthermore, viral diseases have more importance in Tilapia culture and rate of morbidity and mortality were related to age, water temperature and other culture conditions. Some Herpes-like virus, Iridoviral disease (IVD) and Viral Nervous Necrosis (VNN) could be considered as most important viral threats in Tilapia industry in the world. In this article most important infectious (Bacterial and Viral) diseases will be discussed in Iran and abroad.

SPECIFIC SUBTYPES OF ATYPICAL *AEROMONAS SALMONICIDA* REPRESENT A SIGNIFICANT RISK TO 'CLEANER FISH' USED IN NORWEGIAN SALMON FARMING

S. GULLA[1, 2, 3]*, B. KROSSØY[2], Ø. VÅGNES[2], D. COLQUHOUN[1]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*Vaxxinova Norway AS, Bergen, Norway*
[3]*Norwegian University of Life Sciences, Oslo, Norway*

Norwegian salmon farming has in recent years seen a dramatic increase in the use of so-called 'cleaner fish' for bio-control of the salmon louse, *Lepeophtheirus salmonis* (Krøyer). Since 2010, more than ten million wild wrasse (Labridae) have been caught annually for this purpose. Although farming of Ballan wrasse, *Labrus bergylta* (Ascanius), and lumpsucker, *Cyclopterus lumpus* (L.), has been initiated to meet the growing demands, wild-caught wrasse continue to dominate.

Currently, cleaner fish mortalities in salmon farms are high, and atypical strains of *Aeromonas salmonicida* are among the most commonly isolated disease causing agents. Molecular typing (A-layer typing; *vapA* gene sequencing) has revealed that two surface A-layer types of the bacterium dominate in Norwegian cleaner fish and their pathogenic potential towards Ballan wrasse has been verified through infection trials.

We performed a culture- and qPCR survey of *A. salmonicida* prevalence in wild Norwegian wrasse, captive cleaner fish mortalities in salmon farms, and farmed cleaner fish (prior to cage stocking). The survey identified carrier status in ~5% of the wild wrasse studied and ~70% in captive cleaner fish mortalities. No farmed fish prior to cage stocking were *A. salmonicida* positive.

The results suggest that there is an urgent requirement for vaccine development against *A. salmonicida* infection in the cleaner fish species now used in Norwegian aquaculture. Both cleaner fish-associated A-layer types of this bacterium should presumably be included in such a vaccine.

ROLE OF TYPE III SECRETION EFFECTORS OF *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA* IN FURUNCULOSIS IN FISH

F. ORIGGI[1], P. VANDEN BERGH[1], O. BENEDICENTI[3], T. WAHLI[2], H. SEGNER[2], J. FREY[1]*

[1]*Institute of Veterinary Bacteriology, University of Bern, Switzerland,*
[2]*Centre for Fish and Wildlife Health, Vetsuisse, University of Bern, Switzerland*
[3]*Scottish Fish Immunology Research Centre, Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK*

Aeromonas salmonicida subsp. *salmonicida* (*A. salmonicida*), the etiologic agent of furunculosis of salmonid fish, possesses a complex type three secretion system (T3SS) that secretes and translocates into the host cells potent toxins such as the ADP ribosyltransferase exotoxin AexT and several effector proteins that are all determining the virulence of this pathogen. Deletion mutants of the secretion gene *ascV* or the translocation genes *aopB* or *acrV* in a highly virulent *A. salmonicida* resulted in complete loss of cytotoxicity towards Rainbow Trout Gonad Cells (RTG-2) and in full attenuation as shown by infection of rainbow trout (*Oncorhynchus mykiss*). Both, cytotoxicity and virulence in rainbow trout could be restored by *trans*-complementation with the respective secretion or translocation genes. In contrast, the inactivation of the *aexT* toxin gene, keeping the T3SS intact, only resulted in a partial reduction of virulence as shown by a delay in the cytotoxic effect on RTG-2 cells compared to wild type (w.t. *A. salmonicida*).
High-throughput proteomics was used to display the differences between *in vitro* secretome of w.t. *A. salmonicida* and a T3SS-deficient mutant. Results revealed that the effectors AopH, AexT, AopP and AopO as well as Ati2, AopN and ExsE that are known as effectors in other pathogens and the needle subunits Ati1, and AscX are highly abundant and secreted in virulent w.t. *A. salomonicida*, while they are not secreted and only produced at minor amounts by the secretion deficient mutant *A. salmonicida* Δ*ascV*. Hence, the above named effectors all are secreted and regulated by T3SS. Effectors AopH, Ati2, AexT, AopB and AopD were in the top seven most abundant excreted T3SS proteins as analyzed by Matrix Assisted Laser Desorption/Ionization Time Of-Flight (MALDI-TOF) mass spectrometry. EF-G, EF-Tu, DnaK, HtpG, PNPase, PepN and MdeA were moderately secreted via T3SS. Further analyses revealed that several of the T3SS effectors of *A. salmonicida* have potential immune-suppressive activity. This was evidenced experimentally by showing that after infection of rainbow trout with the virulent w.t. *A. salmonicida* strain interferon gamma (IFN γ) was not induced in head kidney, whereas up-regulation was observed upon infection with the non-virulent Δ*ascV* mutant.

APPLICATION OF IN VIVO INDUCED ANTIGEN TECHNOLOGY TO IDENTIFY GENES OF *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA* THAT ARE SPECIFICALLY EXPRESSED IN THE HOST

S. MENANTEAU-LEDOUBLE*, M. EL-MATBOULI

University of Veterinary Medicine, Vienna, Austria

Most microbial pathogens tightly regulate the expression of their virulence factors and only express them in vivo. This is the basis of the in vivo induced antigen technology in which sera from individuals exposed to the pathogen of interest are harvested. These sera are then exposed to in vitro cultures of the pathogen before being used to screen a genomic library expressing proteins from the pathogen. Because the adsorption process removes antibodies targeting antigens that are expressed in cultures, IVIAT is useful to identify genes that are specifically expressed inside the host. In the present study, we applied IVIAT to *Aeromonas salmonicida* subsp. *salmonicida* and identified eight genes based on sequence homology: AopO (an effector protein of the Type 3 secretion system); LamB (a TonB-dependent protein involved in the acquisition of several nutrients, especially iron-compounds); a lactoylglutathione lyase (involved in the resistance against oxidative and pH stress); RNA polymerase sigma factor D (a regulator of gene expression); TonB (providing the energy for the transport of a variety of compounds across the cell membrane); UDP-3-O-acyl-N-acetylglucosamine deacetylase (involved in cell wall synthesis) as well as a two conserved hypothetical proteins. The function of these genes was identified based on literature and further characterized using PSORT, Pfam and Protfun 2. The expression levels of the genes were then compared between bacteria grown in vitro and bacteria from infected rainbow trout tissues using quantitative reverse-transcription PCR. These RT-qPCRs allowed us to confirm that the genes were more expressed inside the host. The results from this study will contribute to further our understanding of the virulence factors in *A. salmonicida* and might suggest interesting targets for the development of new vaccines.

PROTEIN-PROTEIN INTERACTIONS OF TRANSLOCATED EFFECTORS OF THE *EDWARDSIELLA ICTALURI* TYPE III SECRETION SYSTEM

L. DUBYTSKA, R. THUNE*

Louisiana State University, Baton Rouge, Louisiana, USA

The gram-negative bacterial pathogen *Edwardsiella ictaluri* causes enteric septicemia of catfish (ESC), an economically significant disease of farmed-raised channel catfish, *Ictalurus punctatus*. *Edwardsiella ictaluri* is the leading cause of disease loss in the catfish industry, accounting for an estimated 20.2% loss in 2009. We have demonstrated survival and replication of *E. ictaluri* in channel catfish macrophages and have also identified a Type III secretion system (T3SS) that is essential for *E. ictaluri* virulence and intracellular replication. We also identified nine effector proteins encoded in the *E. ictaluri* genome and demonstrated that they are actively translocated from the *Edwardsiella* containing vacuole (ECV) to the host cell cytoplasm by the T3SS. The effect of the translocated proteins on the host cell varies depending on the host target protein and its function. Functions range from intracellular uptake, surface colonization of the cell without uptake, adherence to macrophages and inhibition of phagocytosis, cytotoxicity, vesicular trafficking, programed cell death, up or down regulation of inflammatory cytokines and gene expression. In order to determine the host cell target proteins for the *E. ictaluri* effectors, a yeast-two hybrid system (Y2H) screening was employed. The system depends on the fact that transcription initiation factors are comprised of two domains, a DNA binding domain (BD) and an activation domain (AD). Basically, individual effector fusions to the GAL4 BD (bait) were constructed on pDEST32, while the cDNA fusions to the GAL4 AD (prey) were constructed on pDEST22. The bait and prey plasmids were then transformed into *S. cerevisiae* strain MaV203, which contains single copies of three reporter genes, *HIS3, URA3*, and *lacZ* that are stably integrated at different loci in the yeast genome. The promoter regions of the three reporter genes all contain the upstream activation sequence (UAS) for GAL4. The GAL4 BD/effector fusion protein binds to the GAL4 UAS, and if the bait protein interacts with a protein in the prey library, the GAL4 AD is brought together with the GAL4 BD through the interaction of the two proteins, resulting in transcriptional activation of the reporter genes located downstream from the UAS. Positive interactions are initially detected by selection on plates lacking histidine, so that only cells producing histidine from an activated HIS3 can grow. Replica plating to plates lacking uracil, so that only cells producing uracil from an activated URA3 can grow, will confirm a positive protein/protein interaction. Positive clones are further confirmed by quantitative detection of β-galactosidase activity. To date, putative target proteins have been identified, including the invariant chain of MHC-II (CD74), which is involved in regulation of antigen recognition, the major vault protein, which is involved in regulation of innate immunity, and the 26s protease regulatory subunit 66, which is involved in ATP-dependent degradation of ubiquinated proteins.

EUROPEAN PHARMACOPOEIA REQUIREMENTS FOR FISH VACCINES

C. LANG[1]*, C. LORTEAU[2], Ø. EVENSEN[3]

[1]EDQM, Council of Europe, France
[2]French Agency for Food, Environmental and Occupational Health & Safety, France
Chair of Ph. Eur. Expert Group 15V, EDQM, Council of Europe
[3]Norwegian University of Life Sciences (Norway)

Medicines need to be safe, effective and of good quality, which in an ever-changing environment represents an endless challenge.
The European Pharmacopoeia (Ph. Eur.)(1), which celebrated its 50th anniversary last year, lies at the heart of drug quality standards in Europe. It has the objective of progressively elaborating a single set of specifications for the quality of medicines and their components, for example active substances and excipients, providing the official standards applicable within its member countries and beyond.
This Workshop will open with a presentation on the work, policies and procedures of the Ph. Eur. with a special focus on Ph. Eur. texts covering fish vaccines. It will then be followed by discussions on ways to promote a move towards *in vitro* methods for the potency testing of fish vaccines as stipulated in the Ph. Eur. and the demonstration of, for example, safety with regard to water temperature, the intra-peritoneal injection site reaction, existing potency tests and their suitability (2,3). The Ph. Eur. Group 15V has a continuous focus on 3R aspects and the potential replacement of *in vivo* by *in vitro* potency tests, including fish vaccines.

References
1) "European Pharmacopoeia: Assuring the Quality of Medicines", Lang, C., Vielle, C., Keitel, S.
2) "Towards in vitro methods for potency testing of fish vaccines", Evensen Ø, Lang, C., Brady, A-M., Lorteau, C.
3) "Animal use in the quality control tests for the batch release of vaccines intended for fish", Tout A., Cooney R., Brady A.M., Paterson N.

More information is available on the website: www.edqm.eu.

TOWARDS *IN VITRO* METHODS FOR POTENCY TESTING OF FISH VACCINES

Ø. EVENSEN[1]*, C. LANG[2], A.-M. BRADY[3], C. LORTEAU[4]

[1]*Norwegian University of Life Sciences, Oslo, Norway*
[2]*European Directorate for the Quality Control of Medicines and HealthCare (EDQM), Strasbourg, France*
[3]*Veterinary Medicines Directorate, London, UK*
[4]*French Agency for veterinary medicinal products - French Agency for food, environmental and occupational health safety, Paris, France*

The EDQM, Council of Europe, is organising a workshop session on the "European Pharmacopoeia requirements for Fish Vaccines" at the 17th EAPF Conference. This workshop is aimed at enabling participants to expand their knowledge with the work and procedures of the European Pharmacopoeia (Ph. Eur.) with a special focus on fish vaccines. Here we present the principles and requirements for documentation of safety and efficacy of vaccines for farmed fish laid down in existing monographs. The European Pharmacopoeia Group 15V has a continuous focus on 3R aspects and and the potential replacement of *in vivo* by *in vitro* potency tests, including fish vaccines (1).
Here we present some of the studies documeting that *in vitro* tests can be used for documentation of vaccine efficacy, like for *Aeromonas salmoncida* vaccines intended for use in Atlantic salmon (3, 4, 5). These studies show that the level of circulating antibodies as tested by ELISA methods *in vitro* correlate with level of protection obtained by *in vivo* challenge models. Under given circumstances the *in vitro* show even better resolution than in vivo methods, particularly at minimal deviation from defined potency range (4).
In addition to furunculosis vaccines, scientific reports have also documented that level of circulating antibodies post vaccination against infectious pancreatic necrosis (IPN) of Atlantic salmon, correlate well with protection obtained by in vivo challenge (2). In this latter study, a cut-off level (OD value) was defined that required to obtain protection against mortality.
Together, the referred studies give reason for optimism that *in vitro* can replace current *in vivo* challenge based on mortality as endpoint. These would represent an important step towards replacing current methods in the relevant Ph. Eur. monographs (6) and thereby reduce the number of animals used for batch testing of vaccines for farmed fish.

(1) Midtlyng PJ, Hendriksen C, Balks E, Bruckner L, Elsken L, Evensen O, Fyrand K, Guy A, Halder M, Hawkins P, Kisen G, Romstad AB, Salonius K, Smith P, Sneddon LU. Biologicals. 2011;39(2):117-28
(2) Munang'andu HM, Fredriksen BN, Mutoloki S, Dalmo RA, Evensen Ø. Vet Res. 2013;44:7
(3) Romstad AB, Reitan LJ, Midtlyng P, Gravningen K, Emilsen V, Evensen Ø. Biologicals. 2014;42(2):86-90;
(4) Romstad AB, Reitan LJ, Midtlyng P, Gravningen K, Evensen Ø. Vaccine. 2013;31(5):791-6;
(5) Romstad AB, Reitan LJ, Midtlyng P, Gravningen K, Evensen Ø. Biologicals. 2012 Jan;40(1):67-71.
(6) European Pharmacopoeia Online 8.5: General monograph Vaccines for veterinary use (0062)
4 specific monographs for fish vaccines: Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids (1521) ; Vibriosis (cold-water) vaccine (inactivated) for salmonids (1580) ; Vibriosis vaccine (inactivated) for salmonids (1581) ; Yersiniosis vaccine (inactivated) for salmonids (1950).

INTRAGENOMIC HETEROGENEITY IN THE 16S RRNA GENES OF *FLAVOBACTERIUM COLUMNARE* AND STANDARD PROTOCOL FOR GENOMOVAR ASSIGNMENT

B.R. LAFRENTZ[1]*, G.C. WALDBIESER[2], T.J. WELCH[3], C.A. SHOEMAKER[1]

[1]*United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Aquatic Animal Health Research Unit, Auburn, AL, USA*
[2]*USDA-ARS, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS, USA*
[3]*USDA-ARS, National Center for Cool and Cold Water Aquaculture, Kearneysville, WV, USA*

Genetic variability in 16S rRNA gene sequences has been demonstrated among isolates of *Flavobacterium columnare* and a restriction fragment length polymorphism (RFLP) assay is available for genetic typing this important fish pathogen. Interpretation of restriction patterns can be difficult due to the lack of a formal description of the expected number and sizes of DNA fragments generated for each of the described genomovars. In this study, partial 16S rRNA gene sequences (ca. 1250 bp fragment) from isolates representing each described genomovar and isolates generating unique restriction patterns were cloned and sequenced. The results demonstrated that some isolates contained up to three different 16S rRNA genes whose sequences generate different RFLP patterns due to intragenomic heterogeneity within *Hae*III restriction sites. The occurrence of *Hae*III restriction sites within the portion of the 16S rRNA gene used for typing the *F. columnare* isolates and intragenomic heterogeneity within these sites explained the restriction patterns observed following RFLP analyses. This research provides a standard protocol for typing isolates of *F. columnare* by RFLP and a formal description of the expected restriction patterns for the previously described genomovars I, II, II-B, and III. Additionally, we describe a new genomovar, I/II.

NEW PCR PRIMERS FOR *FLAVOBACTERIA* AND *TENACIBACULUM* SPECIES BASED ON 16S rRNA GENE SEQUENCING

G. GUTTORMSDOTTIR*, S. HJARTARDOTTIR, T. EINARSDOTTIR

The Institute for Experimental Pathology, University of Iceland, Reykjavík, Iceland

Bacterial disease outbreaks annually cause major economic losses in aquaculture worldwide. In Iceland infections of species, of the family Flavobacteriaceae, e.g. *Flavobacterium* spp. and *Tenacibaculum* spp., are increasingly problematic. While the disease symptoms can vary between fish species, these bacteria are generally associated with fin erosion, and responsible for a phenomenon commonly called tail- and fin rot. Rapid bacterial spread and the subsequently emerging disease symptoms, calls for immediate action, i.e. reliable diagnosis of the causative agent followed by a relevant antibiotic treatment.

Until recently, diagnosis of species of Flavobacteriaceae was roughly based on clinical signs of the fish and the bacteria's characteristic yellow colony phenotype. Culture of the bacteria can be difficult, mostly due to overgrowth of contaminating fast growing environmental bacteria. Furthermore, the differentiation of bacterial strains, e.g. *Flavobacterium* spp., is not possible using these techniques. More recently, PCR analyses have been incorporated into the diagnosis of these bacteria. Icelandic *Flavobacterium* strains that had been identified using colony appearance and microscopic examinations were analyzed with nested PCR, using known species-specific primers for the 16S rRNA gene. Furthermore, type strains of *Flavobacterium* and *Tenacibaculum* species were analyzed in the same way.

Nested PCR of type-strains identified the bacteria as *Flavobacterium* and *Tenacibaculum* genera. Analysis of bacteria isolated from diseased Icelandic fish indicates that both *Flavobacteria* and *Tenacibaculum* species are responsible for fin- and tail rot in Icelandic aquaculture. However, the primers were not suitable for a subset of bacteria, which led to misidentification of the infectious agent. The aim of the study is to develop new species-specific PCR tests for known pathogenic bacteria causing tail and fin rot, based on the 16S rRNA gene that can discriminate between species of Flavobacteriaceae.

GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF NORWEGIAN *TENACIBACULUM* ISOLATES RECOVERED FROM SKIN ULCERS IN ATLANTIC SALMON

S. BANG SMÅGE[1, 2]*, Ø. BREVIK[2], H. DUESUND[2], K.F. OTTEM[3], K. WATANABE[1], A. NYLUND[1]

[1]*Fish Disease Research Group, University of Bergen, Norway*
[2]*Cermaq Group AS, Oslo, Norway*
[3]*Cermaq Norway, Nordfold, Norway*

Skin ulcers in sea-reared Atlantic salmon have been reported in Norway since the late 80`s. The disease cause significant economic loss and is a major health and welfare problem in the Norwegian aquaculture industry. The causative agent is assumed to be the bacterium *Moritella viscosa.* In recent years there has been increasing attention regarding the potential role of bacteria in the genus *Tenacibaculum* in causing skin ulcers as they are often found in mixed infections with *M.viscosa* or as the apparent sole agent. Over a three year period, 20 different *Tenacibaculum* isolates have been collected from Atlantic salmon suffering from ulcerative disease at different sites in Northern Norway. Phylogenetic analysis of the 16s rRNA gene shows that these isolates consist of three clusters; a *T.ovolyticum* cluster, a *T.dicentrarchi* cluster, and a third cluster with Norwegian *Tenacibaculum* isolates. A substantial phenotypic and genotypic study of selected field isolates has been performed and the results will be presented.

NORWEGIAN *TENACIBACULUM* ISOLATES CAUSING ULCERATIVE DISEASE IN FIELD AND IN A CHALLENGE STUDY

Ø. BREVIK[1]*, V. VOLD[2], K.F. OTTEM[3], A. NYLUND[1]

[1]*Cermaq Group AS, Oslo, Norway*
[2]*Fish Disease Research Group University of Bergen, Bergen, Norway*
[3]*Cermaq Norway, Nordfold, Norway*

The “winter ulcer” disease has been a problem for the Norwegian salmon farming industry for the last thirty years. The causative agent is assumed to be the bacterium *Moritella viscosa.* However, in recent years there have been increased attention regarding bacteria from the genus *Tenacibaculum* and their potential role in causing ulcerative disease in the Northern parts of Norway. Studies of field outbreaks show that the ulcers are predominantly colonized by bacteria from genus *Tenacibaculum.* In order to clarify the role of Norwegian *Tenacibaculum* isolates in causing ulcerative disease in farmed Atlantic salmon, a challenge study has been performed using two Norwegian *Tenacibaculum* isolates on Atlantic salmon. Results from this study showed that the bacteria were able to reproduce the pathology observed in field without the presence of *M.viscosa*, thereby fulfilling Koch's postulate. Field data and results from the challenge experiment will be compared and consequences discussed.

WHOLE GENOME SEQUENCING OF THE SALMONID PATHOGEN *YERSINIA RUCKERI* ISOLATED OVER 27 YEARS IN TASMANIA, AUSTRALIA REVEALS SLOW EVOLUTION OF TWO DISTINCT GENOTYPES WITHIN THE O1B SEROTYPE LEADING TO LOSS OF MOTILTY AND ENHANCED BIOFILM FORMATION

A.C. BARNES[1]*, J. DELAMARE-DEBOUTTEVILLE[1], R. MORRISON[2], J. CARSON[2]

[1]*The University of Queensland, Brisbane, QLD 4072*
[2]*Department of Primary Industries, Parks, Water and Environment, Launceston, Tasmania 7249, Australia*

The complete genomes of forty-four *Yersinia ruckeri* isolates collected from Atlantic salmon and rainbow and brown trout in Tasmania since 1987 were sequenced by Illumina and/or PacBio RSII sequencing technologies. Core genome comparison based on 4345 single nucleotide polymorphisms (SNPs) revealed two distinct genotypes within the O1b serotype: A dominant genotype was found only in Atlantic salmon and showed very slow evolution between 1987 and 2014, with only 32 SNPs differing between the earliest and latest isolates. A second genotype comprised isolates from rainbow trout and was quite distinct from the salmon genotype, differing by over 4000 SNPs across the core genome. The most recent isolates of this genotype were recovered from infected Atlantic salmon and rainbow trout at a salmonid hatchery and were distinct from, but very closely related to, the earlier rainbow trout isolates. In both genotypes, there has been a loss in swimming and swarming motility over time and more rapid biofilm formation. Complete loss in motility in later isolates (putative "biotype 2") is associated with changes in genes of the flagella operon and is distinct from, and therefore independent of, the evolution of biotype 2 in both Europe and the USA. It is likely there has been parallel evolution of *Yersinia ruckeri* towards less motile isolates in response to higher availability of susceptible hosts and the selective pressure applied as a result of the high immunogenicity of flagellin.

FLAGELLAR REGULATION IN *YERSINIA RUCKERI* DURING INFECTION

A.K. SNYDERAND, T.J. WELCH*

National Center for Cool and Cold Water Aquaculture, Agricultural Research Service/U.S. Department of Agriculture, Kearneysville, West Virginia, USA

The gram-negative Enterobacterium *Yersinia ruckeri* is the etiologic agent of enteric redmouth disease (ERM), a septicemia affecting primarily farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum). Over the past decade, there has been an increase in the prevalence of non-motile variants of *Y. ruckeri* and the appearance of these variants has correlated to ERM outbreaks in previously vaccinated fish. Additional research demonstrated that this phenotype has evolved independently several times in the U.S. and Europe and has had no discernable effect on the virulence of this pathogen. Since flagellin, the structural component of the flagellum, is a potent immune stimulator it is plausible that inappropriate expression of the motility phenotype during the infection process could be deleterious due to flagellin-mediated host recognition. Therefore, mutational loss would ensure against inappropriate flagellin expression. Here, we use quantitative RT-PCR to demonstrate that expression of the flagellin locus *fliC*is repressed during the course of infection and subsequently up-regulated upon host mortality in a motile strain of *Y. ruckeri*.We hypothesize that the repression of flagellin expression during infection occurs in order to evade host recognition. Related bacterial flagellar secretion assembly, and thus motility, is often regulated in response to environmental stimuli through transcriptional regulators that act by modulating expression of the flagellar master regulator *flhDC*. Expression of *flhDC* in turn initiates a cascade of downstream flagellar biosynthetic gene activation. To confirm that *flhDC* regulates flagellar secretion in *Y. ruckeri*, we constructed an isogenic *flhD* knockout mutant. This mutation caused complete loss of the flagellar motility and secretion phenotypes when assessed in culture, suggesting that *flhDC* is an essential positive regulator of flagellar assemblyin *Y. ruckeri*. Further work is necessary to identify putative environmental response-regulator(s) necessary for host sensing and *in vivo* repression of flagellar motility during infection. This research will lead to a better understanding of how *Y. ruckeri* senses and responds adaptively to its host environment, and may lead to the identification of other virulence regulated genes.

SUSCEPTIBILITY OF DIFFERENT LUMPFISH (*CYCLOPTERUS LUMPUS*) FAMILIES TO *VIBRIO ORDALII* INFECTION

M.S.W. BREILAND*, L.-H. JOHANSEN, H.E. MIKALSEN, Ø.J. HANSEN, T.M.K. DRANGSHOLT, A. MORTENSEN

Nofima AS - Norwegian Institute of Food, Fisheries and Aquaculture Research, Tromsø, Norway

The use of different types of wrasse and lumpfish (*Cyclopterus lumpus*) as cleaner fish to combat salmon lice in Atlantic salmon (*Salmo salar* L.) farming has become quite common. In recent years commercial production of lumpfish has increased in Norway, however little is known about the susceptibility to diseases in this species and the potential for disease transmission from lumpfish to salmon and *vice versa*.

Vibrio ordalii has been detected from several outbreaks of vibriosis in farmed lumpfish in Norway in 2012-2014. This bacterium causes wounds and significant losses that may extend over time. The present study was conducted to study the susceptibility to *V. ordalii* in different lumpfish families. In 2014 Nofima started a small scale production towards a breeding program for lumpfish which resulted in 52 families successfully produced. Nineteen of these families that had approximately the same age, were chosen for the *V. ordalii* challenge. The broodstock used to produce the families originated from Finnmark, the northernmost county in Norway.

Lumpfish, approximately 45 fish per family, mean weight 15 gr, were challenged in one tank at 15 °C using our established *V. ordalii* co-habitation challenge model. The challenge isolate was originally from an outbreak of vibriosis in farmed lumpfish in northern Norway. Mortality started 2 days post infection (dpi) in the intraperitoneal injected group and 9 dpi in the co-habitation group. *V. ordalii* were re-isolated from head kidneys of dead fish during the trial. Cumulative mortalities in the different families varied widely (2-98 %) and preliminary results indicate that it could be possible to increase resistance against *V. ordalii* in lumpfish by selective breeding. More comprehensive data from the trial will be presented.

SEROLOGICAL DIAGNOSTICS FOR FISH DISEASES – THE UNDERESTIMATED TOOL

S.M. BERGMANN[1]*, M. MATRAS[2], N.J. OLESEN[3], TH. MORIN[4], J. KEMPTER[5]

[1]*FLI, Insel Riems, Germany*
[2]*NVI Pulawy, Poland*
[3]*DTU, Copenhagen, Denmark*
[4]*ANSES, France*
[5]*Western Pomeranian Technical University Szczecin, Poland*

Serological investigations are common and useful in mammals (1) and poultry (2) especially for long term control of infectious diseases. Serological assays are also developed for several viral fish diseases but only few of them are used for routine surveillance, despite the fact that diseases survivors often become latent carriers with significant antibody response.

A major reason for this is lack of knowledge on the kinetics of the antibody response in fish at various water temperatures. Different assays had been developed these past years e.g. SNT, IFAT and ELISAs for surveillance and diagnosis of VHS, IHN, IPN, SVC or KHVD. Samples were collected either for virological or for a serological investigations only, seldom for both purposes while these two approaches, direct and indirect, can be complementary but also contrary. For e.g. KHVD, where the latent infection of disease survivors is generally accepted, virus and antibodies were found 13 years after the initial disease outbreak (3).

In this study we tested putative carrier fish (rainbow trout) for the presence of KHV as well as specific antibodies against KHV by neutralization tests and ELISAs and examined if virus could be transferred to naïve common carp. Additionally an investigation was proceeded where virus and antibody development were compared in common carp, the native host of KHVD, for a 7 months period.

The study was partly sustained by the EU project "EPIZONE, WP 4.5. KHV-Sero" and by the EMIDA–ERA net project "MOLTRAQ".

References:
(1) Ludwig, H. and Gregersen, J.-P. (1986) rev. Sec. Tech. Off. Int. Epiz. 5 (4), 869-878.
(2) McNulty M.S., Allan G.M. and McCracken R.M. (1985). Infectious laryngotracheitis in Ireland. Irish vet. J. ***39****, 124–125.*
(3) Eide K.E., Miller-Morgan, T., Heidel, J.R., Kent, M. L., Bildfell, R.J., LaPatra, C., Watson, G., and L. (2011) Investigation of Koi Herpesvirus Latency in Koi. J Virol. 85(10): 4954–4962.

DEVELOPMENT OF MOBILE REAL TIME PCR ASSAYS FOR BIOMARKER DETECTION OF SMOLTIFICATION IN ATLANTIC SALMON, (SALMO SALAR)

M.J. MCGOWAN[1]*, S. MACKENZIE[1], N. STEIROPOULOS[2], A. DYKES[3], M. WEIDMANN[1]

[1]*Institute of Aquaculture, University of Stirling, Stirling, UK*
[2]*Europharma Scotland Ltd*
[3]*Fishguard UK*

The Global Atlantic salmon (*Salmo salar*) aquaculture industry has greatly increased in recent years and husbandry and management of the salmon life cycle are constantly improving. One issue is trying to attempt bulk smoltification in a short period induced by feed and subsequent accurate and timely identification of the smoltification of the fish. Due to their anadomous nature it is crucial to transfer salmon from fresh water to sea water during smoltification. Too early can result in high mortality of the fish due to them not being fully adapted to the ocean conditions and increase susceptibility to virus infections. Transferring too late can lead to long term health problems and de-smoltification. The use of mRNA transcripts associated with smoltification have been explored but currently no detection platform to monitor smoltification is available to definitively indicate when smoltification is occuring in salmon. The project aims to transfer two qRT-PCR assay for the smoltification biomarkers NaKATPase and GAPDH to the mobile Smart Cycler system for on site detection. Currently both assays have been successfully developed and transferred onto the mobile Smart Cycler system and are currently awaiting field testing. Both assay showed analytical sensitivities of 100 molecules detected per reaction as well as high efficiencies of 99.7%, SE 0.146 (NaKATPase) and 96.3%, SE 0.384 (GAPDH) Successfully development of the on site biomarker detection assay would provide farmers with quick results on the day of testing.Results would indicate which fish are currently going through smoltification and therefore allow transferring of them to sea water at the correct time, reducing the loss of fish and profit.

DIAGNOSTIC VALIDATION OF THE ORF89 QUANTITATIVE PCR (QPCR) TEST FOR DETECTION OF CYPRINID HERPESVIRUS 3 (CyHV-3)

S. CLOUTHIER[1]*, C. MCCLURE[2], T. SCHROEDER[1], M. LINDSAY[1], S. KHATKAR[1], M. DESAI[1], L. HAWLEY[3], G. LOWE[3], E. ANDERSON[4]

[1]*Fisheries & Oceans Canada, Winnipeg, Canada*
[2]*AquaEpi Research, Charlottetown, Canada*
[3]*Fisheries & Oceans Canada, Nanaimo, Canada*
[4]*Ste Anne, Canada*

The CyHV-3 QPCR assay developed by Gilad et al [Diseases of Aquatic Organisms (2004) 60:179] was validated according to guidelines outlined by the World Organization of Animal Health to assess its fitness as a diagnostic test method. Performance measures included diagnostic sensitivity (DSe) and specificity (DSp) using 264 fish and repeatability (within laboratory) and reproducibility (between laboratory repeatability) using 100 fish, a subset of which were also added to populations created for the accuracy study. Latent class models were used to generate estimates of test accuracy in the absence of a gold standard reference test for CyHV-3 detection. Two other diagnostic methods, virus isolation by cell culture on common carp brain cells and the thymidine kinase conventional PCR (cPCR) assay developed by Bercovier et al [BMC Microbiology (2005) 5:13], were also evaluated in the study.
The original primer and probe sequences were retained for both PCR assays but the parameters of each were modified to improve their analytical performance. Tissue samples for the study were collected from wild common carp (*Cyprinus carpio carpio*) previously exposed to CyHV-3 in Lake Manitoba and commercial koi (*Cyprinus carpio koi*) that were injected with the virus. Three laboratories in Canada participated in the study.
Diagnostic validation revealed that all three tests performed well. DSe and DSp estimates of 99% and 93%, respectively, were generated for both PCR tests. For comparison, DSe and DSp estimates for virus isolation were lower at 90% and 88%, respectively. Repeatability and reproducibility of the QPCR assay were high with 79-97% agreement among all dichotomous test results within and between three laboratories. Measurements of agreement adjusted for chance were in moderate to almost perfect agreement (kappa = 0.5 to 0.9) among samples analyzed within a laboratory. With the exception of one laboratory pairing, there was substantial to almost perfect agreement (kappa = 0.60 to 0.94) among samples analyzed between laboratories. The performance characteristics of the CyHV-3 QPCR test indicate that it is suitable for use as a diagnostic assay for surveillance, presumptive diagnosis and certifying individuals or populations freedom from infection or presence of CyHV-3.

DETECTION OF ANTIBODIES SPECIFIC TO KOI HERPESVIRUS (KHV) BY SERUM NEUTRALIZATION TEST

J. CABON[1], L. LOUBOUTIN[1], J. CASTRIC[1], S.M. BERGMANN[2], G. BOVO[3], M. MATRAS[4], O. HAENEN[5], N. JORGEN OLESEN[6], T. MORIN[1]*

[1]*French Agency for Food, Environmental and Occupational Health & Safety (ANSES), European University of Brittany, Plouzané, France*
[2]*Friedrich Loeffler Institut (FLI), Greifswald, Germany*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie (IZS-Ve), Padova, Italy*
[4]*National Veterinary Research Institute (NVRI), Pulawy, Poland*
[5]*Central Veterinary Institute (CVI) of WUR, Lelystad, The Netherlands*
[6]*Technical University of Denmark, National Veterinary Institute (DTU Vet), Frederiksberg, Denmark*

In the late 90s, koi herpesvirus (KHV) was identified almost simultaneously in Europe, the United States, Israel and Japan in *Cyprinus carpio* and its variety the koi. This virus, classified in the order *Herpesvirales* and the family *Alloherpesviridae*, is responsible for numerous severe outbreaks in farms and natural environment. Mortality generally occurred between 5 and 24 days after infection at water temperature between 16 and 25°C and can reach 80 to 100% in all ages of carp or koi. Latency has been demonstrated for KHV in carp that have recovered from an initial viral infection and virus reactivation can be observed several months after the initial exposure following stress situations. During latency, the virus is present at very low copy number in its host and it is difficult to detect using the molecular methods recommended by the OIE. This could be solved by detecting specific antibodies in the host sera but insufficient data are available to date to consider the use of serological tests as routine screening method for assessing the viral status of carp populations. In this study, the analytical and diagnostic performances of an indirect and non-lethal serum neutralization (SN) test developed in Anses laboratory for the detection of KHV specific antibodies was assessed using 151 sera or plasma from healthy, naturally or experimentally infected carp or koi. The French KHV isolate 07/108b was used efficiently to be neutralized by sera from carps infected with European, American and Taiwanese KHV isolates but no neutralization was observed using sera specific to other aquatic herpesviruses (Chanel Catfish Herpesvirus, Herpesvirus Anguillae, Cyprinid Herpesvirus type 1). Diagnostic sensitivity, diagnostic specificity and repeatability respectively of 95.9%, 98.8% and 99.3% were obtained as well as a compliance rate of 89.9% among six laboratories involved in an inter-laboratory proficiency test of the assay. Neutralizing antibodies were detected in latently infected carp more than 25 months post-infection, with various titres as a function of water temperature. The results suggest that SN test could be used in a close future to improve the epidemiological surveillance and control of KHV disease in Europe.

POINT OF NEED MULTIPLEX REAL TIME PCR DETECTION ASSAY FOR THE DETECTION OF SAV, PRV AND PMCV

M.J. MCGOWAN*, B. LOPEZ-JIMENA, M. WEIDMANN

Institute of Aquaculture, University of Stirling, Stirling, UK

Salmon alphavirus (SAV), Piscinesreovirus (PRV) and Piscine myocarditis virus (PMCV) have a major detrimental impact on the salmon aquaculture industry throughout Europe limiting the growth of this industry. Each virus induces disease with almost identical symptoms and morphological impacts in salmon, rendering distinction through current diagnostic methods extremely difficult, slow and inaccurate. There are presently no rapid detection systems available that can quickly and efficiently identify and distinguish between each of these viruses, with all diagnostic tools currently focusing on PCR screening as well as histology, with samples being sent from fish farms to the laboratory. The process from shipping to analysis and final reporting can take between two days to one week. With high rates of mortality, reduced growth and general impaired animal welfare early diagnosis of these diseases is crucial in preventing and or controlling outbreaks in fish farms. This project aims to develop a multiplex real time polymerase chain reaction (qRT-PCR) assay for detection of all three viruses using LNA TaqMan probes. This multiplex qRT-PCR will then be transferred onto a mobile Smart Cycler system to be used as an on site point of need viral detection platform. To increase the speed of detection on site even further, fish samples will be processed using a Quickgene Mini80 system, a semi automated robot that quickly extracts RNA from tissue samples. Currently 3 individual viral assays have been developed for SAV (subtypes 1-6), PRV and PMCV. The SAV and PRV assays showed analytical sensitivities of 10 molecules detected and the PMCV assay detected up to 100 molecules per reaction. Each assay showed high efficiencies of 96.3%, SE 0.212 (SAV), 95.9%, SE 0.183 (PRV) and 97.1%, SE 0.072 (PMCV). These 3 viral assays will be developed into a multiplex assay to provide quick and reliable on site detection within hours. This would allow on site advice on the next step to reducing viral spread and treatment if viral detection was positive.

WITHDRAWN O-109

THE YELLOWHEAD DISEASE COMPLEX: IT'S COMPLEX

N.J.G. MOODY[1,2]*, P.G. MOHR[1,2], J. HOAD[1,2], L.M. WILLIAMS[1,2], R.O. BOWATER[3], D.M. CUMMINS[1,2], J.A. COWLEY[4], M.STJ. CRANE[1,2]

[1]*CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia*
[2]*OIE Reference Laboratory for Yellowhead disease*
[3]*Department of Agriculture, Fisheries and Forestry, Biosecurity Queensland, Townsville, Queensland, Australia*
[4]*CSIRO Agriculture Flagship, Queensland Bioscience Precinct, St. Lucia, Queensland, Australia*

Prior to 2012 the Yellowhead complex of viruses contained six recognized genotypes (YHV1 to YHV6). Only one, YHV1, is highly pathogenic to shrimp, causing Yellowhead disease (YHD) which is an OIE-listed disease. Of the remaining five genotypes, YHV2 or gill-associated virus (GAV), is the only genotype detected in association with disease in farmed shrimp.

During a disease investigation involving *P. monodon* in Queensland in 2013 anomalous results were obtained using the OIE YHV/GAV Protocol 2 RT-nPCR. A number of different RT-PCR assays generated amplicons for sequence comparisons with representatives of the known YHV-complex genotypes. The analysis showed that the genotype detected in these shrimp was most similar to YHV-complex Genotype 1, although only an 88.4% nucleotide similarity was observed. The newly detected strain has been designated YHV Genotype 7 (YHV7). During the same disease investigation it was found that GAV, enzootic to Queensland, can cross-react with the YHV1-specific primer in the OIE GAV/YHV Protocol 2 RT-nPCR. Due to concerns of false-positive results the OIE Manual for YHD was updated to recommend sequencing of amplicons to confirm the test result.

After the detection of YHV7, an FRDC-funded research project commenced with objectives including determining the prevalence and distribution of YHV7 in *P. monodon* Australia, evaluation of new assays specific for YHV7 and refinement of existing OIE assays for the detection of the different YHV genotypes. During the evaluation of these assays, including a new YHV1-specific TaqMan assay, a further two previously-undetected YHV-complex genotypes were detected in four submissions of unprocessed commodity shrimp imported from China in 2014. Three submissions contained YHV8 and one contained YHV10. A further genotype, YHV9 had previously been reported by Chinese scientists. Each of the three newly-detected YHV genotypes (YHV8, YHV9 and YHV10) shared less than 88% nucleotide similarity with each other and any of the previously described YHV genotypes. Research is currently underway to further refine molecular assays for the specific detection of YHV genotypes and to determine the pathogenicity of YHV7, YHV8 and YHV10 to Australian farmed shrimp.

THE ASSESSMENT OF NEW RAPID DIAGNOSIS KIT FOR DETECTION OF VIRAL NERVOUS NECROSIS (VNN) DISEASE AND COMPARISON WITH HISTOPATHOLOGY AND HEMATOLOGY DIAGNOSIS METHODS IN MULLET FISH OF THE CASPIAN SEA

M.E.J. ZORRIEHZAHRA[1]*, M.R. MEHRABI[1], M. GHIASI[2], G. BOVO[3], M. GHASEMI[4], S.H. KARISIDANI[5]

[1]*Iranian Fisheries Research Organization, (IFRO), Tehran, I.R. Iran*
[2]*Mazandaran Echology Research Center, Sari, I.R.Iran*
[3]*Instituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy*
[4]*Inland Water Aquaculture Research Center, Bandar Anzali, I.R. Iran*
[5]*Islamic Azad University, Bandar Anzali Branch, Bandar Anzali, I.R. Iran*

Unknown mortality have been occurred in wild mullet in the Caspian Sea since 2003 that more important clinical sign was abdominal distention. According to primary study and pilot plan that Viral Nervous Necrosis was confirmed by OIE Reference Laboratory in Italy and Japan, and concerning to complementary research investigation project in 2005, it could be concluded that Viral Nervous Necrosis (VNN) was the main causative agent for disease outbreak in Mullet fish in Southern coastline of Caspian Sea. In continuous this study was conducted to finding new rapid diagnosis method for detection of VNNV in suspected Mullet fish in the Caspian Sea. Consequently about 20 pieces of affected and moribund mullet fish were obtained from Khazar Abad beach of Caspian Sea in Feb.2014. collected sampled were transferred to Aquatic health & Diseases laboratory of Ecology Research Center in Mazandaran province. Blood sampling was done before the death of the fish samples. Also,target tissues such as Brain and Eye were collected for histopathology and then supernatant preparation for new kit examination. All investigations were done according to standard methods and kit application. By survey of obtained results, in histopathology specimens, evidences of inflammation, hyperemia and bleeding and cerebral vasodilatation, accumulation of macrophages (MMC), Cell necrosis and severe vacuolation were observed. Findings in affected captured fish revealed significant decrease ($p<0.05$) in R.B.C, Hb, P.C.V. and (MCHC, g/dl) in infected fish comparison with health fishes. In opposite, (MCV, fl) were more significant increase in infected fish than health fishes but (MCH, pg) was no significant difference between two mentioned groups. Also, total IgM, Protein and Albumin have significant decrease ($p<0.05$) in infected fish comparison with health fishes. Although C3 and C4 revealed numerical decrement but have no significant difference between two groups. In opposite, AST and ALT enzymes were more significant increase ($p<0.05$) in infected fish than health fishes. Obtained results revealed positive correlation between our entire findings in mentioned diagnosis methods but spend time for mentioned kit was less than 20 minutes. So, using of new rapid diagnosis kit could be recommended in order to detection of VNN affected fishes as new valued tool in Monitoring & Surveillance program in the region.

ASSESSMENT OF THE INTRAEPITHELIAL STRUCTURES IN THE PYLORIC CAECA AND INTESTINE OF FARMED FINGERLING AND JUVENILE RAINBOW TROUT *ONCORHYNCHUS MYKISS* (WALBAUM, 1792) INFECTED WITH *SPIRONUCLEUS SALMONIS*

P. NOGUERA[1]*, R. HOPEWELL[2], C. COLLINS[1]

[1]*Marine Scotland Science, Aberdeen, Scotland*
[2]*Dawnfresh Seafoods Ltd, Glasgow, Scotland*

The anaerobic or micro-aerophilic diplomonad flagellates include free-living, commensal and pathogenic species, with representatives in wild and farmed, seawater and fresh water, fish hosts. *Spironucleus salmonis* has been described since the 1920's in the intestinal lumen of farmed rainbow trout *Oncorhynchus mykiss,* and can be especially problematic in young fish. Examination of the intestinal content via fresh smears contributes to a preliminary diagnosis through the observation of the rapidly moving trophozoites. Histological examination of affected fish however is limited in detecting low density infections and in matching clinical moderate or severe cases with high density infection or pathological changes directly associated. Moreover, intra-epithelial structures in trout infected with *S. salmonis* have been reported since the first descriptions of the disease, and observed regularly in both naturally and experimentally infected fish. These structures have usually been associated with the dying host cells, through processes of necrosis and / or apoptosis. While a natural cell turnover in the fish gastrointestinal tract is to be expected, and possibly increased in cases of infection, not all the intra-epithelial structures in *O. mykiss* infected with *S. salmonis* are consistent with dying cells. Evidence of what seems to show a multiplication process has been reported[1]. The suggestion raised in the 1920's about the existence of intra-epithelial stages for *S. salmonis* has typically been disregarded subsequently, even though these structures continue to be observed. However, inter-epithelial and intra-epithelial stages of *Spironucleus* are known in birds (e.g. *S. meleagridis* in partridge), suggesting that the structures in *O. mykiss* could indeed be stages of *S. salmonis*. If the unusual structures in *O. mykiss* are identified as *S.bsalmonis*, this would greatly contribute to the management of the condition potentially helping to prevent clinical infections by earlier accurate detection and control actions. . The current work was designed to thoroughly assess the intra-epithelial structures in clinical and non-clinical *S.bsalmonis* positive fish. Our approach to characterization and identification included histology, ultrastructure, and molecular testing, thereby clarify their role in association with *S. salmonis* infection.

[1]*Intestinal diplomonads in fingerling and juvenile stages of Scottish farmed rainbow trout: Is Spironucleus salmonis being underdiagnosed? P. Noguera, R. Hopewell and C. Collins. Oral presentation at the EAFP –UK meeting, September 2014, Keele-UK.*

TRANCHEM PROJECT - TRANSGENERATIONAL EFFECTS OF A CHRONIC EXPOSURE TO PESTICIDE ON THE IMMUNE SYSTEM IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

M. DANION[1]*, J. CABON[1], L. LOUBOUTIN[1], S. LE FLOCH[2], T. MORIN[1]

[1]*Anses, Plouzané, France*
[2]*Cedre, Brest, France*

The presence of pesticides in surface waters, well documented in North America, Europe, Australia and Asia, is a major concern for human health and environment preservation. Pendimethalin (N-(1-ethyl propyl)-2,6-dinitro-3,4-xylidine) is an herbicide active substance commonly used in terrestrial systems. Previous studies have shown that herbicide has effects on innate and adaptive immunity in rainbow trout, causing a disturbance of several components of the immune system. The TRANSCHEM project aims to determine the direct and indirect effects of a long-term exposure to an environmental concentration of pendimethalin on i) adult physiological stage, based on the assessment of the fish health, immune status and reproductive capability and ii) the offspring of parents chronically exposed to the pesticide, through an estimation of transgenerational effects based on the eggs quality, the early development and the anti-infectious defenses of juveniles.
Adult trouts are exposed daily to the herbicide since May 2013 at a concentration close to 230 ng/L and gave two offsprings born in December 2013 (F1_2013) and 2014 (F1_2014). In addition to the transgenerational exposure, a part of F1_2013 was also exposed directly to the herbicide. Blood and target organs were sampled in different fish groups every four weeks and a viral challenge with infectious hematopoietic necrosis virus (IHNv) was made to assess the overall defense potential.
Hematological parameters were modulated in adults trouts during the spawn and transgenerational effects were measured in the F1_2013 due to a chemical transfer in eggs and sperm. While the transgenerational exposure has generated a greater sensitivity of offspring to the virus, the direct exposure to the herbicide from the larvae stages has strengthened their overall defense potential. To go further, other infectious challenges with sleeping disease virus (SDv), infectious pancreatic necrosis virus (IPNv) and IHNv were performed on the F1-2014. As before, transgenerational effects were measured but in an opposite way between pathogens; while the contaminated F1 was more sensitive to the IHNv challenge, it was more resistant to SDv. Several innate and adaptive immune parameters in the two offsprings will be analyzed to better understand and differentiate direct and transgenerational impacts of pendimethalin in rainbow trout.

HARMFUL EFFECTS ON AFRICAN CLAWED FROG (*XENOPUS LAEVIS*) REPRODUCTION AS EXPRESSION OF HIGH WATER PHOSPHATES LEVELS

M. IANNACCONE[1]*, F. SPANÒ[2], G. POSTIGLIONE[3], M. CAMPOLO[4]

[1]*Centro Veterinario il Mondo degli Animali Esotici, Via S. Martino 67R, 16131 Genoa, Italy and Consiglio Nazionale delle Ricerche, Istituto di Biofisica, Via de Marini 6, 16149 Genoa, Italy*
[2]*Consiglio Nazionale delle Ricerche, Istituto di Biofisica, Via de Marini 6, 16149 Genoa, Italy*
[3]*Centro Veterinario il Mondo degli Animali Esotici, Via S. Martino 67R, 16131 Genoa, Italy*
[4]*Zoo delle Maitine, Contrada delle Maitine 1, 82020 Pesco Sannita Benevento, Italy and Centro Veterinario Einaudi, Via N. Colajanni 23/A, 70125 Bari, Italy*

European amphibian population parallels a worldwide decline mostly related to habitat loss, introduction of exotic species, diseases and water quality decrease as a consequence of their sensitivity to environmental changes. Recent investigations suggest that eutrophic conditions may be associated with frog reproduction problems, nevertheless to the authors knowledge, water quality criteria settled for amphibians different species do not currently exist. In this note we summarize our data about water phosphate (PO_4^{3-}) concentrations and their role in hypofertility in Xenopus laevis. Subjects of this study were two different groups of African Clawed Frog (tot: 22 subjects) showing hypofertility: Group A- 14 females Xenopus laevis kept in the National Research Institute of Genova (Italy) in 4 isolated tanks (40-80 L) Group B- 8 subjects (6 females and 2 males) housed in a private collection in 4 isolated tanks (120 L). Both facilities were equipped with isolated biological filtration systems and controlled water temperature (A: 20 °C; B: 22-24°C). In order to identify the primary cause of hypofertility animals were repeatedly subjected to veterinary visits, fecal parasitological exams, ultrasound scans and microbiological analysis of oocytes. Water quality analysis (temperature, pH, ammonia NH^3, nitrite NO^{2-}, nitrate NO^{3-}, phosphates PO_4^{3-}, water hardness, alkalinity) and filtration systems functionality were tested twice a week. Management changes were carried out (e.i. water changes schedule: 30% total volume/ 3 times a week, then 20% total volume/ twice a week), Veterinary tests did not highlight any sign of pathology, results showed good water quality, except for high levels (>5 mg\L) of phosphates. After two months of management improvements phosphate concentrations decreased (PO_4^{3-} < 2 mg/L) contemporaneously morphological and physiological characteristics of oocytes improved. This is the first report that identify a correlation between high levels of PO_4^{3-} and reproduction diseases in Xenopus laevis. When water PO_4^{3-} decreased fertility in African clawed frogs improved, evidencing an involvement of phosphates concentration in reproductive efficiency. Further studies are needed to narrowly define this correlation and to analyse additionally variables in order to fully understand amphibian reproduction.

EFFECTS OF THE JELLYFISH *CYANEA CAPILLATA* INTOXICATION OF ATLANTIC SALMON SMOLT (*SALMO SALAR* L.)

M.D. POWELL*, T. DALE, Å. ÅTLAND

Norwegian Institute for Water Research, Thorøhlens gate 53D, Bergen 5006, Norway

The lions mane jellyfish *Cyanea capillata* is one of the most common coastal and ocean species of jellyfish often growing to large size and forming moderate swarms in coastal regions. The jellyfish can get caught in fish farming cages, tentacles can break off and the fish become intoxicated by the nematocysts. In this study Atlantic salmon smolts were exposed to 5 and 2.5 g L^{-1} of minced jellyfish in duplicate tanks for 2 hours and subsequently recovered over a 4 week period in flow-through seawater. Fish showed acute reaction to exposure including rapid erratic swimming, and lateral flexing behaviours as well as flashing and hyperventilation. Gills showed acute lesions with extensive epithelial separation, congestion and haemorrhage, within 24h, focal inflammatory responses including filamental hyperplasia was evident which gradually resolved over the recovery period. Even by 4 weeks post exposure, focal "nodules" of hyperplasia involving 2-4 lamellae were still observed. Blood chemistry responses indicated acute respiratory responses including hyperventilation and ionic compromise within 24h of exposure then subsequent recovery after 4 days. These studies indicate an acute effect of short-term exposure of Atlantic salmon smolts to this jellyfish species with lesions persisting for several weeks following intoxication.

ABIOTIC FACTORS AND MIXED BACTERIAL INFECTION AS CAUSE OF MORTALITY IN CAGE REARED LENA STURGEON, *ACIPENSER BAERI*

A.V. KAZARNIKOVA[1]*, H.V. SHESTAKOVSKAYA[2], M. GALEOTTI[3], A.V. TRISHINA[4], A.A. TURCHENKO[5]

[1]*Southern scientific center of RAS, 41, Chekhov st., 344006 Rostov-on-Don, Russia*
[2]*CPS laboratory of fish diseases, 49, Oborona st, 344002 Rostov-on-Don, Russia*
[3]*University of Udine, Department of Food Sciences, Veterinary Pathology Section, Via Sondrio 2, 33100 Udine, Italy*
[4]*Rostov-on-Don Anti-Plague Institute, 117/40, Gorki st., 344002 Rostov-on-Don, Russia*
[5]*Fisheries Research Agency, 665, Gorki st., Koysug, Rostov region, Russia*

Lena (Siberian) sturgeon, *Acipenser baerii,* was introduced into aquaculture in southern Russia at last decade. This species has been the subject of artificial rearing in European Russia since 1969 and in Europe since 1981. The intensification of sturgeon rearing, especially in cages, can result in poor water quality due to its contamination by metabolic products and uneaten food. At the same time the deterioration of the environment decreases the resistance of fish and, as a consequence, bacterial diseases can emerge. Bacteria in the genus *Aeromonas, Vibrio* and *Edwardsiella* belong to this group of microorganisms. Particularly, *A. hydrophila* and *A. sobria were* identified as the causative agents of diseases that resulted in fish mortality.

Mortality episodes in 2-year-old Lena sturgeons (*Acipenser baeri*) occurred from the beginning of July until the end of September 2013 in a commercial fish farm in southern Russia. Water samples were collected to evaluate physical/chemical properties. Water pollution by sulphates (625.0-730.0 mg/l) and chloride (986.0-1003.0 mg/l) was detected, together with an increase of the mineralization rates (2.8-2.9 times the normal ratio). Fifteen fish were sampled for haematological, parasitological, microbiological analysis. The changes in red blood cells consisted of erythrocyte agglutination, poikilocytosis, anisocytosis, and development of anaemia. The white blood cells changes were characterized by increased number of band neutrophils, monocytes and by a decrease of lymphocytes and segmented neutrophils. Sixty-seven different bacterial isolates were identified in water (32) and fish (35). Microorganisms belonging to the genus — *Aeromonas*, *Vibrio* and *Edwardsiella* were detected. Histological changes in spleen, liver and heart of fish appeared as congestion, haemorrhages, scattered necrotic areas and severe lymphocyte depletion. Ziehl-Neelsen acid-fast stain of the liver sections showed the presence of numerous bacteria exhibiting morphology and characteristics similar to those of mycobacteria.

Acipenser baerii inhabits the basins of all large Siberian rivers. According to the data of Alekin (Alekin, 1948) the indexes of water mineralization in this area are lower in several orders of magnitude than those registered in the water where the investigated sturgeon were cage cultured. The life cycle of Lena sturgeon take place in fresh water, and only in rare cases it encounter brackish water in estuarial areas.A long exposition of fish to unfavourable abiotic factors such as high mineralization and ion content of the water might have caused an immune dysfunction and reduced the resistance of A. *baerii* sturgeon to multiple bacterial infections.

HOST-PARASITE INTERACTIONS IN BLOOD FLUKE INFECTIONS OF TUNA

B. NOWAK[1]*, A. BRIDLE[1], Y. PENNACCHI[1], M. POLINSKI[1], S. SHIRAKASHI[2]

[1]*University of Tasmania, Launceston, Australia*
[2]*Kinki University, Japan*

Blood flukes from genus *Cardicola* have a significant effect on commercial production of Southern Bluefin Tuna (SBT) and Pacific Bluefin Tuna (PBT). *Cardicola forsteri* and *C. orientalis* infect SBT and *C. orientalis* and *C. opistorchis* infect PBT. *C. forsteri* and *C. opistorchis* adults are usually present in the heart, whereas *C. orientalis* adults are found in gill arteries. The two species may co-infect the same individual and produce numerous eggs which accumulate in the host gills resulting in host death. In PBT blood fluke infections are a problem predominantly among 0 year old fish and the juveniles are treated with praziquantel which effectively eradicates the adult flukes. Re-infections of blood flukes occur after the first drug treatment, thus repeated treatments are required to minimise the mortality. SBT are infected after transfer to ranching grounds and praziquantel treatment is used to minimize the effect of infection on the fish. Immune response to blood flukes was investigated at gene and protein level. SBT produces antibodies against *C.forsteri* and their titre and seroprevalence are closely associated with the pattern of prevalence and abundance of the blood fluke infection. Infected PBT showed an organ specific transcriptive immune response associated with the severity of infection. In heart, significant increase in IgM, MHC2, TCRb, and IL-8 transcription was observed in hearts of infected fish relative to uninfected controls; whereas in the gills only IgM was induced by infection. IgM transcription was highly correlated to the relative abundance of *C. orientalis* DNA in the gill samples.

TRANSCRIPTOMIC AND ULTRASTRUCTURAL CHANGES OF THE ATLANTIC BLUEFIN TUNA *THUNNUS THYNNUS* (LINNAEUS, 1758) GILL EPITHELIUM INFECTED WITH *DIDYMOSULCUS KATSUWONICOLA* (POZDNYAKOV, 1990)

Ž. TRUMBIĆ[1*], J.B. TAGGART[2], J.E. BRON[2], I. BOČINA[3], I. MLADINEO[4]

[1]*University Department of Marine Studies, University of Split, Split, Croatia*
[2]*Institute of Aquaculture, University of Stirling, Stirling, UK*
[3]*Faculty of Natural Sciences, University of Split, Split, Croatia*
[4]*Institute of Oceanography and Fisheries, Split, Croatia*

In the Atlantic bluefin tuna *Thunnus thynnus* (Scombridae), didymozoids, considered one of the most taxonomically complex digenean families, inhabit all conceivable niches. They encyst mostly in pairs in a connective tissue capsule of varying size and thickness formed mutually by parasite and the host. *Didymosulcus katsuwonocila* (syn. *Didymocystis wedli*) is the most abundant didymozoid species infecting farmed *T. thynnus* with gills as a predilected site of infection. In most cases infections terminate without any gross pathology, characterised by a significant decrease of cysts per host after couple of months of tuna rearing, however under certain conditions inflammatory and necrotic changes in the gill tissue may be induced. This dynamics is explained as the net result of tuna physiology and specifics of the rearing environment, making didymozoid infection an interesting model for parasite-host interaction study. The main goal of our study was to assess the response of tuna gill tissue parasitised by *D. katsuwonicola* at the trasncriptomic level and compare it to the ultrastructural changes inferred by transmission electron microscopy (TEM). Punch biopsies were collected from infected and uninfected *T. thynnus* gills at harvest, at the end of a two-year rearing cycle. Samples were prepared for transcriptomic profiling by DNA microarrays and TEM according to standardized protocols. *T. thynnus*-specific custom 15K Agilent oligo microarray was employed in this study. The transcriptional profiling revealed moderate gene regulation in both directions with fold changes ranging from -10.38 to 4.56 (N=768 statistically selected features). Pathway analyses based on KEGG sets showed the perturbations of components of innate immunity, complement and coagulation cascades, as well as endocrine, digestive and nervous functional pathways. Micrographs showed a heterogeneous cell population at the host-parasite border. Numerous transiting vesicles were recorded in didymozoid's tegument in direct contact with host's loose collagen connective tissue capsule comprising fibrocytes, fibroblasts, eosinophilic-granulated mast cells, eosinophils and plasma cells. The capsule encompassed a nerve composed of three neuronal axons, numerous anastomosing capillaries and was overlaid with multilayered squamous epithelium abundant with mucous goblet cells. The findings elucidate the utility of a molecular and ultrastructural approach in a host-parasite interaction study.

EXPRESSION OF PROINFLAMMATORY CYTOKINES IL-1β, TNFα1 AND TNFα2 INDUCED BY PAMPs AND GILL PARASITIC INFECTIONS IN THE ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) PERIPHERAL BLOOD LEUKOCYTES AND GILLS

I. LEPEN PLEIĆ[1], I. BUŠELIĆ[1], Ž. TRUMBIĆ[2], I. BOČINA[3], M. ŠPRUNG[3], I. MLADINEO[1]*

[1]*Institute of Oceanography & Fisheries, Split, Croatia*
[2]*University Department of Marine Studies, Split, Croatia*
[3]*Faculty of Natural Sciences, University of Split, Split, Croatia*

Parasites infecting vascularised tissues, especially gills, are in general quickly detected by both locally secreted and circulating immunity components, causing stronger or weaker inflammatory reaction. Atlantic bluefin tuna *Thunnus thynnus* is one of the most valuable aquaculture species in the Mediterranean, and interestingly, no economically threatening parasitoses have been reported in adult fish in the Adriatic or Mediterranean aquaculture. Moreover, a decrease in parasite populations is usually observed by the end of the rearing cycle. In order to shed the light on the immunity mechanisms underlying Atlantic bluefin tuna reactions against different pathogen types (bacteria, viruses, parasites) we studied expression profiles of three major cytokines; IL-1β, TNFα1 and TNFα2 in three different scenarios: 1) in peripheral blood leukocytes (PBL) during *in vitro* stimulation with lipopolysaccharid (LPS) and polyinosinic-polycytidylic acid (Poly I:C) mimicking bacterial and viral pathogens; 2) in PBL during *in vitro* stimulation with didymozoid *D. katsuwonicola* (Digenea) and siphonostomatoid *P. appendiculatus* (Copepoda) antigens; and 3) at the site of *D. katsuwonicola* and *P. appendiculatus* parasitation. It resulted that the Atlantic bluefin tuna IL-1β and TNFα2 were induced in LPS/Poly I:C-stimulated PBLs and PBLs treated by parasite-derived antigens, as well as in parasites infected gills. Induction of Atlantic bluefin tuna pro-inflammatory cytokines IL-1β and TNFα2 by PAMPs, *D. katsuwonicola*- and *P. appendiculatus*-derived antigens, as well as during natural infection of two parasites, suggests they play an important role in inflammation and host defence against wide array of pathogens, in contrast to TNFα1. Targets' expressions in general followed congruent pattern in parasites TA-stimulated PBL (*in vitro* model) and in host tissue (*in vivo* model), diverging only in respect to parasite species.

HISTOPATHOLOGICAL ALTERATIONS INDUCED BY GILL AND SKIN PARASITIC INFECTIONS IN THE ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) AND ITS INNATE RESPONSE MODELED *IN VITRO*

I. LEPEN PLEIĆ[1], I. BUŠELIĆ[1], J. HRABAR[1], M. ŠPRUNG[2], I. BOČINA[2], I. MLADINEO[2]*

[1]*Institute of Oceanography & Fisheries, Split, Croatia*
[2]*Faculty of Natural Sciences, University of Split, Split, Croatia*

For the farming purposes, juvenile Atlantic bluefin tuna (ABT) *Thunnus thynnus* are fished in the South Adriatic and transferred into farming cages, introducing along an abundant and diverse parasite community. Throughout the farming process, it was observed that most parasites taxa decrease, not constituting a serious economic threat for the aquaculture. Didymozoid trematode *Didymosulcus katsuwonicola* and siphonostomatoid copepods *Pseudocycnus appendiculatus* and *Brachiella thynni*, are all gill/ skin parasites frequently parasitizing tuna, whose populations conspicuously decline during the farming cycle. In order to shed the light on the immunity mechanisms underlying Atlantic bluefin tuna reaction to these three species, we studied expression profiles of three major cytokines; IL-1β, TNFα1 and TNFα2 in peripheral blood leukocytes (PBL) during *in vitro* stimulation by didymozoid and siphonostomatoids antigens. As an additional record to support molecular results of the later scenario, a pathohistological analysis of parasitic infections was performed on semi- and ultrathin sections of infected gill filaments. Resulting induction of the Atlantic bluefin tuna pro-inflammatory cytokines IL-1β and TNFα2 by parasite-derived antigens was evidenced, while cellular response inferred by TEM differed in respect to parasite species. Cellular innate response to the digenean showed rather chronic character, resulting with parasite encapsulation in connective tissue, and mast cells, eosinophils, goblet cells, and occasional rodlet cells found at the site of infection. In contrast, copepods attached to the gill/ skin epithelium by clamping, caused direct tissue disruption with undergoing necrotic or apoptotic processes, and extensive proliferation of rodlet and goblet cells. In all cases, it seems that the presence of moderate inflammatory reaction fails to seriously endanger parasites existence, and that other factors should be relevant in its decrease.

VIBRIO SPLENDIDUS AND *VIBRIO AESTUARIANUS* ASSOCIATED WITH MORTALITY EVENTS IN SHELLFISH IN SCOTLAND

H. STAGG*, J. BLACK, E. MUNRO, U. MCCARTHY

Marine Scotland Science, Aberdeen, Scotland, UK

The Scottish shellfish industry was worth approximately £8.9 million in 2013, with a total production of 6953 tonnes. The two main species farmed are the blue mussel, *Mytilus edulis* and the Pacific oyster, *Crassostrea gigas*. Disease surveillance is carried out under an approved risk-based surveillance scheme and in addition to this, diagnostic investigations are undertaken during unexplained mortality events. Between 2010 and 2015, there was a total of 17 diagnostic investigations at shellfish sites. Using *atpA* sequencing, *Vibrio splendidus* was identified in a case of increased mortality among blue mussels in 2010. The following year, *V. aestuarianus* was isolated from moribund oysters. Subsequent investigations have not produced conclusive diagnoses although *Vibrio* spp. were isolated in all cases. As there has been an increasing number of reports throughout Europe of *V. splendidus* and *V. aestuarianus* being associated with mortality events in shellfish, further identification of the Scottish vibrio isolates was carried out. Multi-locus sequence analysis was performed on fifty isolates from shellfish diagnostic investigations. These bacteria were all previously identified as *Vibrio* sp. based on biochemical tests. Bacteria isolated from haemolymph of unaffected mussels were also analysed to compare bacterial populations. Three housekeeping genes were selected for sequencing and subsequent phylogenetic analysis, *recA, rpoB* and *atpA*. Isolates closely matching *V. splendidus* LGP32 (based on Genbank sequences) were isolated from affected mussel spat during five mortality events where up to 50% mortality was reported. *V. aestuarianus* was also isolated a second time from Pacific oysters, in this case from both affected and unaffected animals.

PROTEOMIC ANALYSIS OF *VIBRIO ORDALII* REVEALS IRON-REGULATED PROTEINS RELATED TO VANCHROBACTIN AND PISCIBACTIN-LIKE SIDEROPHORES

P. RUIZ[1, 4]*, M. BALADO[2], C. HIDALGO[3], A.E. TORANZO[2], M.L. LEMOS[2], R. PAREDES[3], R. AVENDAÑO-HERRERA[1, 4]

[1]*Universidad Andrés Bello, Viña del Mar, Chile*
[2]*Universidad de Santiago de Compostela, Santiago de Compostela, Spain*
[3]*Universidad Andrés Bello, Santiago, Chile*
[4]*Interdisciplinary Center for Aquaculture Research (INCAR), Chile*

Vibrio ordalii is a Gram-negative bacterium that is the etiological agent of atypical vibriosis in Chilean salmon farms, although its pathogenic mechanisms are not yet fully understood. Since the amount of free iron available to an invading pathogen within a host is extremely low, the ability to obtain it has been shown to be a critical factor for the pathogenicity of most microorganisms. It is known that *V. ordalii* is able to produce siderophores when growing under iron limited conditions, but so far few information is available about their nature. In the present study we used a proteomic approach to identify iron-regulated proteins in *V. ordalii* strains isolated from farmed salmon in Chile. For this purpose three *V. ordalii* strains were grown in iron-rich and iron-limited media, and the profiles of total and outer membrane (OM) proteins were compared using 1D and 2D electrophoresis approach. The differentially expressed proteins were selected and identified by peptide mass fingerprinting (PMF) analysis and MASCOT searches. The three strains studied showed similar protein profiles. From each strain we could identify 13-15 proteins that were iron-regulated since they were detected only when the strains grew under iron limitation. Three of those proteins are high molecular mass proteins that were identified as non-ribosomal peptide synthetases (NRPS) involved in siderophore synthesis: a protein of 311 kDa that showed a 99% identity to VabF, a NRPS described in *V. anguillarum* that is required for vanchrobactin synthesis, and two proteins of 270 kDa and 224 kDa that showed 52% and 57% identities to Irp1 and Irp2, two NRPS involved in the synthesis of piscibactin, a siderophore described in *Photobacterium damselae* subsp *piscicida* that is encoded within a high pathogenicity island. In addition, two putative TonB-dependent OM receptors were also identified: a protein of 71 kDa that showed 99% identity to the *V. anguillarum* heme receptor HutS, and a protein of 60 kDa that showed 66% identity to the piscibactin receptor FrpA. The results suggest that pathogenic *V. ordalii* strains could produce vanchrobactin and/or piscibactin-like siderophores. Results of an *in silico* analysis of *V. ordalii* genomes available in GeneBank are congruent with these obsrvations and show that *V. ordalii* genome harbors two independent siderophore systems with high homology to vanchrobactin and piscibactin synthesis systems. The elucidation of the precise role of the described proteins and the identification of the actual siderophores being produced by *V. ordalii,* togeher with their possible connection with virulence for salmon, are currently under way. Overall, our results provide new insights about the biology of this fish pathogen.
FIA PYT-2013-0014, FONDAP/CONICYT 15110027 and CONICYT Doctoral Scholarship no. 21110146.

FUNCTIONAL AND MUTATIONAL ANALYSIS OF *cpsG* AND *cpsE* IN THE CAPSULAR OPERON OF *STREPTOCOCCUS INIAE* IMPLICATE *cpsG* IN SEROTYPE SWITCHING AND VACCINE ESCAPE AND REVEAL A DISTINCT OPERON ARRANGEMENT FOR WZY-DEPENDENT CPS BIOSYNTHESIS IN *S. INIAE*

A.C. BARNES*, C.M. HEATH, C.M. GILLEN, M.J. WALKER

The University of Queensland, Brisbane, Queensland 4072, Australia

Streptococcus iniae causes septicaemia and meningitis in marine and freshwater fish wherever they are farmed in warm-temperate and tropical regions. Although serotype specific, vaccination with bacterins is largely successful, and vaccine failure occurs only occasionally through emergence of new capsular serotypes. Previously we showed that mutations in vaccine escapes are restricted to a limited repertoire of genes within the 20-gene capsular polysaccharide (cps) operon: *cpsG,* a UDP-galactose 4-epimerase, has three sequence types whilst *cpsE* was more highly variable with eight amino acid sequence types. To elucidate the roles of these genes in cps biosynthesis and capsular composition, we first expressed each of the 3 sequence types of *cpsG*, and assayed their epimerase activity in a coupled reaction, revealing difference in activity between two of the three sequence types. Next, we prepared isogenic knockout mutants of *cpsG* and *cpsE* by allele exchange mutagenesis. Deletion of *cpsG* resulted in changes in colony morphology, but also significantly decreased the galactose content relative to glucose in the capsular polysaccharide as determined by GC-MS. Interestingly, deletion of *cpsE* resulted in no detectable phenotype in terms of growth, colony morphology, antigenicity or cps production/composition. Further proteomic analysis revealed that *cpsE* is not a glycosyl-transferase, as previously reported based on annotation by synteny. Multiple domain similarity searches based on hidden Markov models suggest *cpsE* is a dehydratase and may act on the UDP-sugar complex, prompting a re-evaluation of the operon structure and annotation. The relatively high mutation rate coupled with the lack of discernible phenotype in the deletion mutant suggests that *cpsE* is not a requirement for cps biosynthesis and may be under a process of reductive evolution which will result in its eventual loss or deletion. This is consistent with recovery of strains with defective truncated *cpsE* from infected fish.

MEMBRANE PROTEOME PROFILE OF *STREPTOCOCCUS INIAE* FROM TWO DIFFERENT HOSTS BY SHAVING APPROACH

F. EL AAMRI[1]*, D. PADILLA[2], F. REAL[2], L. ROMÁN[3], J. BRAVO[3], S. DÉNIZ[2], F. ACOSTA[3]

[1]*Institut National de Recherche Halieutique "(INRH) Centre Specialisé en Aquaculture, Maroc*
[2]*Instituto Universitario de Sanidad Animal (IUSA). Universidad de Las Palmas de Gran Canaria. Arucas, Spain*
[3]*Grupo de Inmunología Veterinaria y Producción Animal. Universidad de Las Palmas de Gran Canaria. Arucas, Spain*

Streptococcus iniae are hemolytic Gram-positive cocci that are a major pathogen in fish. Most proteins involved in bacterial virulence are extracellular, cell wall- or membrane-associated, facilitating direct interaction between bacteria and host cells promoting bacterial colonization, entry into host cells or host cell death.
The shaving approach was applied to define the surfacomes of *S. iniae* isolated from two different hosts: fish and human. This approach successfully identified 54 secreted and surface proteins, including several proteins involved in cell wall synthesis and transport of solutes, as well as proteins with yet unknown function. These proteins highlight as interesting targets for further investigation in the interaction between *S. iniae* and its environment. The results reported in this study have shown a first analysis of experimental surface proteome of *S. iniae* from different origins.

FIRST REPORT OF *STREPTOCOCCUS PARAUBERIS* FROM TURKEY

Z.H. BEKTAS[1]*, F.B. UCAR[2], S. SAVAŞER[1]

[1]*Fisheries Research Station Directorate Egirdir-ISPARTA, Turkey*
[2]*Department of Biology, Basic and Industrial Microbiology Section, Faculty of Science, Ege University, İzmir, Turkey*

Streptococcus parauberis is an alpha-hemolytic gram-positive coccoid bacterium belonging to the Streptococaceae family. This bacterium cause streptococcosis is a major disease in cultured fish due to intensification of aquaculture and causes significant economic losses in fish farm industry. In our study we isolated a total of 34 lactic acid bacteria from wild fish (*Cyprinus carpio*, *Sander lucioperca*, *Carassius gibelio*) of Lake Eğirdir and from cultured fish farm (*Onchorhynchus mykiss*) of Isparta province in Turkey. From the isolated lactic acid bacteria 25 of were identified as *Streptococcus parauberis*, 2 of as *Vagococcus* sp., 2 of as *Lactococcus garvieae* and 5 of as *Lactococcus lactis* by culture-based, biochemical test and molecular techniques. In this study, we reported the first identification of *Streptococcus parauberis* from wild fish in Lake Eğirdir and from cultured fish of fish farm of Egirdir-Turkey.

OUTBREAKS OF *PSEUDOMONAS ANGUILLISEPTICA* IN CULTURED AYU *PLECOGLOSSUS ALTIVELIS* IN JAPAN

Y. SHIMAHARA[1]*, I. KIRYU[1], Y. KAWATO[1], T. NISHIOKA[2], K. YUASA[1], S. MIWA[1], T. KAMAISHI[3], N. OSEKO[4], M. HOSAKA[5], T. NAKAI[6]

[1]*National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan*
[2]*National Research Institute of Aquaculture, Fisheries Research Agency, Saiki, Oita, Japan*
[3]*Fisheries Agency, Tokyo, Japan*
[4]*Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Sapporo, Hokkaido, Japan*
[5]*Akita Prefectural Fisheries Promotion Center, Oga, Akita, Japan*
[6]*Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan*

Ayu *Plecoglossus altivelis* is an important freshwater fish for aquaculture industry in Japan. In February to March 2013, mortality of ayu occurred in a rearing facility located in northeastern area of Japan. The mortality rate was 86% in 2 months, and the affected fish showed hemorrhage on operculum and skin. In this study, histological and bacteriological studies were carried out to determine the etiological agent. Furthermore, experimental infection was conducted to clarify the pathogenicity of isolate. In histological study, a number of rod-shaped bacteria were observed in inner skin, muscle, connective tissue, meninges, heart, vessels, and spleen. A total of 3 strains (H2432-03, 04 and 07) were isolated from the kidney and muscles on TSA and NA at 20^{o}C. The isolated bacteria were Gram-negative and identified as *Pseudomonas anguilliseptica* by biochemical reactions and 16S rRNA gene analysis. The LD_{50} of H2432-03 strain was $1.7x10^{3}$cfu/fish, indicating the high pathogenicity to ayu. In Japan, infection with *P. anguilliseptica* was reported in Japanese eel, ayu, striped jack and black seabream in 1970s to 1990s. The 16S rRNA gene analysis revealed that the strains isolated from ayu in this study showed 100% identity with *P. anguilliseptica* 1123/5 strain, which was isolated from gilthead seabream in Europe (Genbank accession no. X99541) (seabream type), but not with *P. anguilliseptica* NCMB1949^{T} and SH82424, which were isolated from eel and ayu in 1970s to 1980s in Japan (eel type). It was confirmed that the seabream type of *P. anguilliseptica* was responsible for high mortality outbreaks in farmed ayu in Japan.

CHARACTERIZATION OF *PISCIRICKETTSIA SALMONIS* FROM CHILEAN AND CANADIAN SALMONIDS

A. OTTERLEI[1], D. JENSEN[1], H. DUESUND[2], Ø.J. BREVIK[2], I. SOMMERSET[3], P. FROST[3], J. MENDOZA[4], P. MCKENZIE[5], A. NYLUND[1], T. UTNE[1], S. JOHANNESSEN[1], P. APABLAZA[2]*

[1]*University of Bergen, Bergen, Norway*
[2]*Cermaq Group AS, Bergen, Norway*
[3]*MSD Animal Health, Bergen, Norway*
[4]*Cermaq Chile, Puerto Montt, Chile*
[5]*Cermaq Canada, Campbell River, BC, Canada*

Salmonid rickettsial septicemia (SRS), caused by *Piscirickettsia salmonis*, is an important disease for the Chilean aquaculture resulting in substantial mortalities and antibiotic use. The current knowledge about *P. salmonis* is limited in aspects as characterization of strains from different sources or pathogenicity. The objective of this study was to describe both phenotypic and genetic differences among Chilean and Canadian isolates of *P. salmonis*. The strains were retrieved from Atlantic salmon and rainbow trout and several phenotypic tests were applied: different agar media, growth temperature, antibiotic susceptibility, and biochemical methods. The type strain of *P. salmonis* LF-89, isolated from Coho salmon, was also included in the study. Genetic analyses comprised phylogeny of 16S rDNA-ITS, concatenated housekeeping genes and multiple locus sequence typing (MLST). The results showed that the strains were fairly homogenous phenotypically, with the exception of some Chilean isolates. Clustering of isolates based on genotyping suggested a geographical distribution among the isolates, whereas no host specificity was found. The results from characterization propose a putative new species of *Piscirickettsia* sp. or a subspecies of *P. salmonis.* In addition, two selected Chilean isolates were tested in challenge experiments in Atlantic salmon postsmolts, by cohabitation, both in the presence and absence of sea lice *Lepeopththeirus salmonis*, and by intraperitoneal injection. The results showed differences in mortalities and in tissue tropism for both isolates. The presence of *L. salmonis* does not appear to affect the severity of the infection with *P. salmonis*.

ARE BROWN TROUT AND RAINBOW TROUT TWO OF A KIND? A SPECIES COMPARISON OF INFECTION BY *TETRACAPSULOIDES BRYOSALMONAE*

T. WAHLI*, C. BAILEY, H. SCHMIDT-POSTHAUS, H. SEGNER, N. STREPPARAVA

Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Switzerland

Tetracapsuloides bryosalmonae is the causative agent of Proliferative Kidney Disease (PKD), which is considered an emerging disease of freshwater salmonids in Europe and North America in farmed and wild fish populations. *T. bryosalmonae* is able to infect both rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), however, as yet, a comparative study characterizing differences between the two species in susceptibility to parasite infection and parasite development in the host is not available. An influence of water temperature on disease development is suggested. We therefore aimed to determine differences in the parasite infection prevalence and intensity between brown trout and rainbow trout hosts and between two infection temperatures.

Brown trout and rainbow trout were kept at two different water temperatures each (i.e. 12 and 15°C) and exposed during a limited time period (1 hour) to the identical quantity of *T. bryosalmonae* spores of the same genetic composition. Infection intensity and parasite development were followed by analyzing kidney samples by qPCR for a period of 100 days post exposure (p.e.).

Results indicate a different development of the parasite with respect to fish host species. In brown trout kept at 15°C, *T. bryosalmonae* could be detected for the first time 7 days p.e., whereas in rainbow trout parasites were not found before day 15 p.e.. No difference between species was found at 12°C, where infection was detected after 15 d.p.e. Throughout the experiment prevalence of infected fish and parasite intensity was greater in brown trout than in rainbow trout in the respective temperature group. The species differences were accentuated by temperature, with temperature influencing infection prevalence, parasite proliferation and intensity in brown trout but not in rainbow trout. The results point to important differences in *T bryosalmonae* infection between brown and rainbow trout; future studies will have to show if this is related to different virulence of the parasite for the two salmonid species or to species differences in the fish immune response.

CHARACTERIZATION OF GENES RESONSIBLE FOR SPOROGENESIS OF *TETRACAPSULOIDES BRYOSALMONAE IN* BROWN TROUT (*SALMO TRUTTA*) AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

G. KUMAR, S. MENANTEAU-LEDOUBLE, M. EL-MATBOULI*

Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria

Tetracapsuloides bryosalmonae is an enigmatic myxozoan parasite which causes proliferative kidney disease in salmonids. Spores of *T. bryosalmonae* develop in the kidney of infected brown trout *Salmo trutta* and are released via urine to infect the bryozoan *Fredericella sultana* colonies. In the present study, brown trout and rainbow trout were infected with the spores of *T. bryosalmonae*, released from mature sacs of parasite from laboratory-infected *F. sultana* colonies and maintained at 16.5±1 °C under laboratory conditions. The posterior kidneys of both infected and non-infected fish were sampled at different time points post infection (6, 8, 10, and 12 wpe). Infection in individual kidney was confirmed by quantitative real-time PCR and immuno-histology. The transcriptomes of kidneys of infected and non-infected brown trout and infected rainbow trout were compared by suppressive subtractive hybridization (SSH). The differentially expressed transcripts resulting from SSH were cloned, transformed, tested and validated using dot blot screening. The positive clones were sequenced, analyzed and validated by qPCR in kidney samples from both fish species at different time points post infection. Significant changes in the transcription levels of the genes of interest were observed in the kidney samples during the *T. bryosalmonae* development. This study suggests that differential expression of host anti-inflammatory, humoral immune and endocytic pathway responses, signal transduction activities, cell proliferation and cell growth processes do not inhibit the development of intra-luminal sporogonic stages of the European strain of *T. bryosalmonae* in brown trout but suppress it in rainbow trout.

PROLIFERATIVE KIDNEY DISEASE (PKD) IN SALMONIDS: DRIVEN BY INCREASED WATER - TEMPERATURE?

N. STREPPARAVA*, H. SCHMIDT-POSTHAUS, H. SEGNER, T. WAHLI

Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Switzerland

Proliferative Kidney Disease (PKD) is a condition caused by *Tetracapsuloides bryosalmonae*, a parasite belonging to the Myxozoa and shifting between a vertebrate (fish) and an invertebrate host (bryozoan). This parasite is wide-spread in freshwater systems of the northern hemisphere and can cause high mortalities among salmonid populations. PKD is considered an emerging disease since both incidence and severity of the disease in fish populations substantially increased over the last decades. As water temperatures of Swiss rivers experienced a significant rise over the last 20 years, this has led to two possible scenarios contributing to the recent emergence of PKD in wild Swiss brown trout (*Salmo trutta*): A) climate-related increase of water temperatures is favoring the spread of the invertebrate host, the bryozoans, and with this also of the parasite and B) higher water temperatures promote parasite infection and development in the fish host. Here, we tested the second scenario.
Brown trout were exposed to *T. bryosalmonae* and kept at two temperatures (12°C and 15°C). Kidney samples were taken routinely and prevalence and parasite intensity were assessed by qPCR. Additionally, water samples were collected to estimate start of spore shedding from infected fish and length of the shedding period.
Fish showed different patterns of parasite development at the two experimental temperatures: At 15°C, the infection was detectable earlier than at 12°C, the infection prevalence and infection intensity were higher, proliferation of parasites was faster, and, finally, release of spores occurred earlier when compared to fish kept at 12°C.
In this experiment, higher temperature enhanced parasite proliferation in the fish thereby promoting an earlier release of parasite spores. This may lead to prolonged exposure of bryozoans to infective spores. Remarkably, although the parasite cycle is likely to have evolved at lower water temperatures, as they are characteristic for brown trout habitats; higher water temperatures promote parasite infection and proliferation in the fish host. Parallel investigations are ongoing to examine the temperature effect on parasite dynamics in wild fish populations.

DEVELOPMENT OF AN *IN VITRO* MODEL TO STUDY SALMONID INNATE IMMUNE RESPONSES TO *PARAMOEBA PERURANS*

I. CANO, A. BAYLEY, S. GUNNING, R. PALEY*

Cefas, Weymouth Laboratory, Weymouth, UK

An *in vitro* model was tested to study the host innate immune response to *Paramoeba perurans*, the causative agent of amoebic gill disease (AGD). Rainbow trout (RTgill-W1 cell line) cells grown in transwell plates were infected with $4x10^3$ *P. perurans* in SW. Additional cells were inoculated with SW only as a negative controls. Amoebae were observed mainly attached on the RTgill-W1 monolayer 3 h post inoculation (pi). Within 6 h pi small foci of cytophatic effect (CPE) were observed. No growth of the amoebae was observed during the assay. Cells were harvested in triplicate at 0, 1, 3, 6, 24, 48, 72 and 96 h pi. The copy number of *P. perurans* 18S rRNA gene was quantified by Taqman qPCR. A maximum number of 18S rRNA gene copies was observed at 3 h pi (3.2×10^7 copies) which decreased 1 log during the 4 consecutive days of the test (to $1x10^6$ at 96 h pi).

The expression of three genes involved in the rainbow trout immune response to AGD was compared between infected and uninfected cells. Genes investigated were interleukin-1 beta (IL-1ß), inducible nitric oxide synthase (iNOS) and major histocompatibility complex class I (MHCI). Rainbow trout elongation factor (EF) 1α was used as a reference gene. IL-1ß was significantly up regulated in cells inoculated with *P. perurans* from 3 h pi to the end of the assay, with a peak at 48 h. There was no clear pattern of differential expression of the iNOS gene between infected and uninfected cells. Relative MHCI gene expression in infected cells was down-regulated from 6 h to the end of the assay.

This *in vitro* model has proved to be a promising tool to study host response to AGD, however more development is needed to provide optimal growth conditions for *P. perurans*.

AMOEBIC GILL DISEASE CHALLENGE AND INFECTION IN ATLANTIC SALMON AND RAINBOW TROUT UNDER LABORATORY CONDITIONS – INFECTION DYNAMICS AND COHABITATION INFECTION

M.D. POWELL[1]*, L. ANDERSEN[2], A. LITLABØ[3], S. NYLUND[4], S. BLINDHEIM[5], A. NYLUND[5]

[1]*Norwegian Institute for Water Research*
[2]*Industrialaboriet (ILAB) Univeristy of Bergen*
[3]*Kobbevik og Furuholmen Oppdrett AS*
[4]*Pharmaq Analytiq*
[5]*Univeristy of Bergen*

Amoebic Gill Disease (AGD) is rapidly becoming a significant health issue regard Norwegian Atlantic salmon and rainbow trout production. The present study aimed to investigate the development of disease, represented by hyperplasia of the gill filament epithelium, and colonization of the gill by *Paramoeba perurans* in both Atlantic salmon and in rainbow trout by direct challenge (adding trophonts to the water) and by cohabitation. Direct challenge resulted in a concentration dependent increase in both amoebae numbers (by PCR) as well as gill pathology (histopathology). In cohabitant Atlantic salmon analysis of the concentration of *Paramoebae* in the water (using PCR) demonstrated discrete periods of shedding that appeared to correspond to specific levels of pathology and amoebae density on the gills. In rainbow trout, a similar trend was observed, but, like shown by other studies, rainbow trout are less susceptible to infection and develop disease more slowly with amoebae numbers being lower both on the gill and within the water column during infections.

PROTEOMICS TO STUDY AMOEBIC GILL DISEASE IN FARMED ATLANTIC SALMON *SALMO SALAR*

M. MARCOS-LÓPEZ[1, 2]*, H.D. RODGER[2], I. O'CONNOR[1], M. BRACELAND[3], D. ECKERSALL[3], E. MACCARTHY[1]

[1]*Galway-Mayo Institute of Technology, Galway, Ireland*
[2]*Vet-Aqua International, Oranmore, Ireland*
[3]*University of Glasgow, Glasgow, United Kingdom*

Atlantic salmon *Salmo salar* farming is a dynamic, growing and worldwide industry. Infectious diseases represent one of the most significant threats for the success and sustainability of aquaculture. Amoebic gill disease (AGD) is currently one of the most significant diseases in all Atlantic salmon producing countries. The aetiological agent, the protozoan parasite *Neoparamoeba perurans*, colonizes the gills inducing a proliferative reaction that manifests as respiratory problems and mortalities if left untreated. AGD has the potential to severely impact on the sustainability of the sector due to fish welfare, stock losses and cost of treatment. In attempt to address one of the major knowledge gaps, this study investigates, using classic proteomics techniques, the salmon host response to *N. perurans* and the pathogenesis of the disease. For the first experiment presented here, gills (including mucus) were chosen as the target organ. An *in-vivo* bath AGD challenge was carried out using naïve Atlantic salmon (average weight 80g) and cultured *N. perurans*. Sequential gill samples were taken from non-infected and infected individuals at 1, 2, 3, 8, 15 and 21 days post infection. Positive samples included both sub-clinical and clinical stages of different severities. Samples were snap frozen at collection and stored at -80°C until required. Samples were homogenised in a detergent based buffer and analysed using 2D gel electrophoresis (2DE) with 11cm IPG isoelectric focusing strips. Differently expressed peptides were identified through digital analysis (NonLinear SameSpots) of the 2DE gels, excised and analysed by mass spectrometry for protein identification. Proteomics represents a novel and complementary approach to the study of AGD in comparison to the previously described histopathology and gene expression, in particular the identification of peptides involved in the response to the disease. The ultimate aim of this project is to use the data generated to identify potential biomarkers and alternative treatments strategies to mitigate AGD in farmed Atlantic salmon. The host gill proteome response will be presented and discussed in relation to disease status.

DIFFERENTIALLY EXPRESSED PROTEINS IN GILL AND SKIN MUCUS OF ATLANTIC SALMON (*SALMO SALAR*) AFFECTED BY AMOEBIC GILL DISEASE

V. VALDENEGRO-VEGA[1]*, P. CROSBIE[1], A. BRIDLE[1], M. LEEF[1], R. WILSON[2], B. NOWAK[1]

[1]*Institute for Marine and Antarctic Sciences, University of Tasmania, Launceston TAS, Australia*
[2]*Central Science Laboratory, University of Tasmania, Hobart TAS, Australia*

The external surfaces of fish, are covered by mucus, which forms a thin interface between the organism and water. Amoebic gill disease (AGD) is a parasitic condition caused by *Neoparamoeba perurans* that affects salmonids worldwide. This disease induces excessive mucus production in the gills. Gill and skin mucus samples were obtained from Atlantic salmon (*Salmo salar*), which were infected with *N. perurans* on four successive occasions. NanoLC tandem mass spectrometry (MS/MS) was used to identify proteins in gill and skin mucus of Atlantic salmon affected by AGD. A total of 186 and 322 non-redundant proteins were identified in gill and skin mucus respectively, and 52 gill and 42 skin mucus proteins were classified as differentially expressed in mucus samples from AGD-affected fish. By generating protein-protein interaction networks, some of these proteins formed part of cell to cell signalling and inflammation pathways, such as C-reactive protein, apolipoprotein 1, granulin, cathepsin, angiogenin-1. In addition to proteins that were entirely novel in the context in the host response to *N. perurans*, our results have confirmed the presence of protein markers in mucus that have been previously predicted on the basis of modified mRNA expression, such as anterior gradient-2 protein, annexin A-1 and complement C3 factor. This first proteomic analysis of AGD-affected salmon provides new information on the effect of AGD on protein composition of gill and skin mucus. Future research should focus on better understanding of the role these components play in the response against infection with *N. perurans*.

PRODUCTION SCALE RAS-FARMING: A FOLLOW-UP STUDY ON WATER PARAMETERS, FISH HEALTH AND WELFARE

A.M. ERIKSSON-KALLIO[1]*, K. PELKOLA[2], T. KIURU[3]

[1]*Finnish Food Safety Authority Evira, Production Animal and Wildlife Health Research Unit*
[2]*Veterinary Bacteriology Research Unit*
[3]*Natural Resources Institute, Finland*

Fish health and welfare parameters in recirculation aquaculture systems are still poorly documented. Finnish Food Safety Authority Evira, Arvo-Kala Ltd. and VTT Technical Research Centre of Finland conducted a two-year research project called "On-line water parameter monitoring and fish health in production scale RAS", aiming to lead to a better understanding of causal relationships between water quality and fish health in production scale RAS. The study, funded by the European fisheries fund, was performed in a rainbow trout fingerling and food fish producing production scale recirculation farm. In this study, certain fish health parameter changes such as histopathological changes in gills and inner organs, bacteriological isolations and parasitological findings were documented together with welfare parameters such as fin lesions. These findings were compared with water parameters (oxygen, temperature, pH, ammonia, nitrite, nitrate, carbon dioxide and suspended solids) measured. Results from one production cycle (10-800g, time period of 6 months) will be presented.

INFLUENCE OF A DENITRIFICATION - MEMBRANE - REACTOR ON THE BACTERIAL MICROFLORA IN A RECIRCULATING AQUACULTURE SYSTEM

V. JUNG-SCHROERS[1]*, M. ADAMEK[1], A. BOLEY[2], A. KORSHUN[2], D. STEINHAGEN[1]

[1]*Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2]*Institute for Sanitary Engineering, Water Quality and Solid Waste Management, University of Stuttgart*

Recirculating aquaculture systems offer the opportunity to keep high numbers of fish without the need of high amounts of fresh water due to recirculation and filtration of tank water. Problems can occur if the amount of nitrate or bacteria in the water increases.
In the present study a reactor with an integrated denitrification membrane was installed to a recirculation aquaculture system. As control an identical system without such a reactor was used. In both systems carp (*Cyprinus carpio*) were kept at a water temperature of 25°C. The bacterial microflora was analyzed in tank water, biofilms of tanks and filters and on skin and gills of carp kept in both systems. Samples were taken before starting the reactor and 24, 52 and 126 days after the start of the reactor. The amount and the composition of bacteria were analyzed by using classical biological techniques and molecular techniques like qPCR. Bacterial diversity was measured by Denaturing Gel Electrophoresis (DGGE). To examine the stress level for fish, cortisol was measured in the water of the tanks and in blood of carp.
Cortisol levels increases continuously in water and blood of carp from the control system. In contrast cortisol levels remained lower and more stable in the system with the denitrification membrane. The total amount of bacteria in the recirculated water increased especially in the control system. Two month after the start of the reactor a five times higher amount of bacteria could be detected compared to the system with installed reactor. Additionally on the gills of carp from the control system we could also find a higher bacterial load compared to fish from the system with reactor.
Overall it could be shown that the reactor with a denitrification membrane could decrease the total amount of bacteria in the tank water of a recirculating aquaculture system. The diversity of bacteria was higher in the system with installed reactor and the carp in this system seemed to have less stress. Therefore the usage of a reactor with denitrification-membrane can have a positive influence on fish health and welfare.

FISH-FRIENDLY PROPHYLAXIS/DISINFECTION IN AQUACULTURE: LOW CONCENTRATION OF PERACETIC ACID IS STRESS-FREE TO THE CARP (*CYPRINUS CARPIO*) AFTER REPEATED APPLICATIONS

D. LIU[1,2]*, L.-F. PEDERSEN[3], D. L. STRAUS[4], T. MEINELT[1]

[1]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*
[2]*Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt University, Berlin, Germany*
[3]*National Institute of Aquatic Resources, Hirtshals, Denmark*
[4]*Harry K. Dupree—Stuttgart National Aquaculture Research Center, Stuttgart, USA*

Application of peracetic acid (PAA) at low concentrations has been proved to be a broad-functional and eco-friendly prophylaxis/disinfection method against various fish pathogens. Therefore, regular applications of low concentration PAA is sufficient to control (potential) pathogens in recirculating aquaculture systems (RAS). However, there is lack of knowledge whether application of low concentration PAA can affect fish welfare. We evaluated in the present study whether repeated applications of low concentration PAA could induce continuous stress to the carp. The stress response was estimated by the increase of water cortisol released from the carp. The results showed that the increase of water cortisol became less significant and occurred earlier along repeated applications of low concentration PAA. It indicates faster but reducing stress response of the carp. We conclude that peracetic acid at low concentration is an adaptable stressor to carp, and regular applications should cause no chronic stress. Regarding the fish welfare in aquaculture, low concentration of PAA is suitable to be applied regularly in recirculating aquaculture systems.

IMPACT OF STOCKING DENSITY ON STRESS PARAMETERS AND THE IMMUNE STATUS OF PIKEPERCH (*SANDER LUCIOPERCA*)

K. KNOPF*, M.E. GARCIA, S. KRÄMER, T. PREUER, W. KLOAS

Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

The stocking density of fish in aquaculture is of critical importance for animal welfare. The aim of this study was to investigate the stress and immune status of pikeperch in a recirculating aquaculture system, in response to different stocking densities (7, 28, 46 and 65 kg/m³). In this context, possible chronic stress was examined by means of growth parameters, spleen and liver index, and the blood parameters cortisol, glucose, lactate, total protein, hematocrit and hemoglobin. To describe the immune status, the respiratory burst activity (potential killing activity, PKA), lymphocyte proliferation and lysozyme activity in the plasma were determined. The heterogeneous growth performance and feed conversion ratio, in addition to the plasma levels of cortisol and immune parameters, indicate an increased level of chronic stress in pikeperch at high stocking densities.
The physiological response to sorting and handling was examined for pikeperch maintained at 28 and 65 kg/m³. The time course of blood parameters shows that the acute stress response was less pronounced in pikeperch maintained at the higher stocking density, and has subsided independent of stocking density after approximately 7.5 h. However, even 48 h after the stress event, PKA of pikeperch, maintained at 65 kg/m³, was significantly lower compared to pikeperch maintained at 28 kg/m³. These results indicate that acute stress might be better tolerated at lower stocking densities.

ULTRA-DEEP SEQUENCING OF VHSV ISOLATES CONTRIBUTES TO UNDERSTANDING THE ROLE OF VIRAL QUASISPECIES IN VIRUS VARIABILITY

A.A. SCHÖNHERZ[1], N. LORENZEN[1]*, B. GULDBRANDTSEN[1], B. BUITENHUIS[1], K. EINER-JENSEN[2]

[1]*Aarhus University, Tjele, Denmark*
[2]*QIAGEN AAR, Aarhus, Denmark*

The high mutation rate of RNA viruses enables the generation of a genetically diverse viral population, termed a quasispecies, within a single infected host. This high in-host genetic diversity enables an RNA virus to adapt to a diverse array of selective pressures such as host immune response and switching between host species. The negative-sense, single-stranded RNA virus, viral haemorrhagic septicaemia virus (VHSV), was originally considered an epidemic virus of cultured rainbow trout in Europe, but was later proved to be endemic among a range of marine fish species in the Northern hemisphere. To better understand the nature of a virus quasispecies on the evolutionary potential of VHSV, a deep-sequencing protocol specific to VHSV was established and applied to 4 VHSV isolates, 2 originating from rainbow trout and 2 from Atlantic herring. Each isolate was subjected to Illumina paired end shotgun sequencing after PCR amplification and the 11.1 kb genome was successfully sequenced with an average coverage of 0.5-1.9 x 10^6 sequenced copies. Differences in single nucleotide polymorphism (SNP) frequency were detected both within and between isolates, possibly related to their stage of adaptation to host species and host immune reactions.

VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV): ON THE SEARCH FOR DETERMINANTS IMPORTANT FOR VIRULENCE IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

N.J. OLESEN[1]*, H.F. SKALL[2], J. KURITA[3], K. MORI[3], T. ITO[3]

[1]*Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark*
[2]*Aarhus University, Faculty of Agricultural Sciences, Aarhus, Denmark*
[3]*National Research Institute of Aquaculture, Fisheries Research Agency, Mie, Japan*

A goal for research on VHS virus in the past decade has been to understand how low virulent virus isolates becomes highly virulent and to pinpoint single determinants that can tell if an isolate is virulent for rainbow trout or not. Focus was primarily on the viral envelope glycoprotein (G) as this protein alone can induce neutralising antibodies and protective immunity. It has been shown that the G-protein is important for virus entry into the host but for virus replication the viral nucleocapsid protein appears to be a very important player. VHSV genotype specific MAbs were previously produced in order to establish a fast and low cost genotyping system for VHSV isolates. In this process, we encountered a number of unpredicted reactions with various VHSV isolates. Some MAbs were able to discriminate between rainbow trout virulent and non-virulent isolates within the same sub-genotype. By assessing the epitope specificity of these MAbs together with cloning and full-length genome sequencing of viruses of various virulence putative virulence markers were identified. Variable regions of the viral nucleocapsid (N) protein i.e. aa 118-123 for Genotype III and aa 43-46 and aa 168 for Genotype Ib and only few amino acid substitutions hereby appear to be of high significance for virulence and replication in rainbow trout.
In vitro studies revealed that replication of non- or low virulent virus are reduced in the rainbow trout cell line RTG-2 cell with 3-4 log reductions when compared to virulent isolates. While no reduction of non- virulent viruses was observed in EPC or BF-2 cell lines.
Infection trials by immersion with the virulent GIII and GIb isolates indicate that the time of exposure to rainbow trout has a significant effect on the virulence to the fish, indicating that the innate immune system is efficient at reducing virus replication of these medium virulent viruses during short exposure.
In conclusion MAbs have shown to be efficient for phenotypic characterization of VHSV isolates in combination with *in vitro* studies on fish cell lines and might in future prove to be efficient for fast and low cost discrimination between isolates of different virulence.

SPECIFIC POSITIONS IN THE 3´-UTR REGION OF VHS VIRUS DICTATE VIRULENCE *IN VITRO* AND *IN VIVO*

S.-H. KIM[1], T.C. GUO[1], V. VAKHARIA[2], Ø. EVENSEN[1]*

[1]*Norwegian University of Life Sciences, PO Box 8146 Dep., 0033 Oslo, Norway*
[2]*University of Maryland Baltimore County, 701 East Pratt Street, Baltimore 21202, USA*

Viral hemorrhagic septicemia virus (VHSV) of the genus Novirhabdovirus and the family Rhabdoviridae belongs to the order Mononegavirales. It has a linear non-segmented negative strand (NNS) RNA genome of 11 kb which encodes the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), RNA polymerase (L), and nonstructural NV gene. The 3´ and 5´ temini of the VHSV genome contains 11-nucleotide-long, conserved sequences that have an unusually high A/U content and they are complementary. Such conserved, complementary sequences are also found in other members of
the order Mononegavirales. Studies of vesicular stomatitis virus (VSV, genus Vesiculovirus in the family Rhabdoviridae), have shown that the initiation of transcription and replication likely occurs at two different sites in the 3'-region of the virus. With the purpose to study the importance of nucleotides in the 3'-UTR of VHSV in replication of novirhabdoviruses, we used reverse genetics and a site-directed mutagenesis of selected residues at the 3'-terminus with resulting mutant viruses. For the obtained mutated clones growth kinetics and in vitro real-time cytopathogenicity were assessed and showed that the order of two nucleotides in position 4 and 5 (A4G5) at the 3'-terminus directly affects growth kinetics in vitro. The permutated mutant A4G-G5A strain had a reduced positive-strand RNA synthesis efficiency (51% of what was observed for wild-type virus; WT) at 48 hrs post-transfection and complete cytopathic
effect in susceptible fish cells was delayed by 70h compared to the WT-VHSV. Further, the A4G-G5A strain was used to challenge zebrafish with resulting reduced pathogenicity, 54% lower end-point mortality compared to WT-VHSV virus. From these studies, we have shown that specific residues in the 3'-UTR of VHSV have a promoter function important for virus transcription and these residues are essential in the sense that they modulate the virus virulence in cells (in vitro) and lower pathogenicity in susceptible fish.

INACTIVATION OF VHSV BY INFILTRATION AND SALT UNDER EXPERIMENTAL CONDITIONS

H.F. SKALL[1, 3]*, C. JØRGENSEN[2], N.J. OLESEN[3]

[1]*Aarhus University, Aarhus, Denmark*
[2]*DHI Hørsholm, Denmark*
[3]*National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark*

At the moment the only legal method in Denmark to sanitize wastewater from fish cutting plants is by infiltration. To evaluate the inactivation effect of infiltration on VHSV an experimental examination was initiated. A column packed with gravel as top- and bottom layer (total of 22 cm) and a mid layer consisting of dug sand (76 cm) was used for the trial. Over a period of 18 h 3.9 x 10^{10} $TCID_{50}$ VHSV was supplied to the column, where after normal tap water was supplied for the rest of the trial period, in total 7 days. During the 7 days samples for virological examination were taken. The sampling was most intensive in the period where the risk of VHSV breaking through the column was highest. The sensitivity of the virological examination was 13.9 $TCID_{50}$/ml and no virus was isolated. A reduction of VHSV > 4 log in the outlet water was seen. This experiment suggests that infiltration can be a valuable method to sanitize VHSV infected water. Changes in temperature, pH, earth types in the area used for infiltration etc. may change the virus reduction, though.
As some of the fish cutting plants are also smoking rainbow trout fillets, the question arose whether a brine solution will inactivate VHSV. In order to answer this question a small trial was set up. VHSV and NaCl was added to cell culture medium with 10% foetal bovine serum, in order to mimic a “dirty” environment, to obtain from 1.9% to 20.9% NaCl and kept in the dark at 4°C. Samples were titrated after 5 min, 1 h and 20 h. No reduction in titre was observed in any of the samples.

VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS LEVELS IN WASTE WATER PRODUCED DURING GUTTING AND FILLETING PRE-CLINICALLY INFECTED RAINBOW TROUT

C. JOINER*, R. PALEY, B. OIDTMANN

Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, UK

There are a number of circumstances when infected fish showing no signs of disease may be slaughtered and processed for human consumption. These fish can be exported from infected areas to uninfected areas if eviscerated, and may be further processed. This may constitute a risk of pathogen spread.

Waste water production from gutting and filleting fish in processing facilities was mimicked to investigate potential levels of viral haemorrhagic septicaemia virus (VHSV) released from infected fish. Market size rainbow trout (*Oncorhynchus mykiss*) were exposed by immersion to VHSV, for 4h at $1x10^5$ $TCID_{50}$ ml^{-1}. Pools of 4 fish, before onset of clinical signs, were eviscerated and the carcasses rinsed in 1 L of water, the kidneys of the fish were scraped into this waste water; in addition, the same fish were filleted and rinsed in 1L of water. Six out of 11 of the resultant water samples were then homogenised in a blender, and all samples were centrifuged and separated. This was to mimic the waste water separation processes within a processing facility. Viral titres in the different fractions of water samples produced were then analysed.

Waste produced from both gutted and filleted pre-clinical fish d6 and d8 post exposure contained high levels of virus. Viral titres in kidney samples taken before processing were consistently higher than in the muscle of the same fish (geomean titres of $2.41x10^8$ compared to $2.94x10^5$ $TCID_{50}$ g^{-1} tissue). Viral titres in the tissue in the waste water produced from gutting or filleting the fish and the supernatant from the same process were similar for both gutted and filleted fish. The geomean titres for all sample fractions were between $6.8x10^5$ and $2.03x10^7$ $TCID_{50}$ g^{-1} tissue. Titres were lower in the supernatant, $8.16x10^3$ to $6.63x10^4$, if observing the titre ml^{-1} in the 1L sample.

This data provides information relevant to undertake risk assessments for imports and spread of VHSV, following a hypothetical introduction of the pathogen, and could be used to inform authorisation requirements for fish processing facilities subject to control measures.

NOVEL VIRAL INFECTIONS IN ATLANTIC SALMON (*SALMO SALAR* L.)

H. SIGURÐARDÓTTIR*, B.Þ. BRAGASON, S. GUÐMUNDSDÓTTIR

The Institute for Experimental Pathology, University of Iceland, Reykjavík, Iceland

The aim of the project was to establish and evaluate diagnostic methods for Piscine reovirus (PRV) and Piscine myocarditis virus (PMCV) two recently defined RNA viruses that infect Atlantic salmon and screen for their presence in Icelandic material. These diseases were first identified in Norway in the late 20th century, in net-pen fish farming. Recently, they have been diagnosed on farms in some countries by the North Atlantic but have never been suspected in Icelandic fish farming. PRV can induce heart and skeletal muscle inflammation and symptoms appear 5-9 months after transfer to sea. PMCV induces cardiomyopathy syndrome and symptoms appear after 12-18 months in sea. Both infections can cause considerable mortality.
Samples were collected from wild Atlantic salmon returning for spawning and from farmed fish reared in a land-based farm and net-pens in seawater. Tissue samples from heart, kidney and gills, average size 30-35 mg, were collected in a tube containing 600 µl RLT lysis buffer. The RNeasy Mini Kit (Qiagen) was used for RNA extraction and the RNA was then used in "One Step RT-qPCR". The reaction parameters, primers and probes were according to published methods. The reference gene used was the elongation factor 1 alpha (ELF1A) gene.
PMCV was not detected in any of the samples from the three groups. PRV screening showed a prevalence of 21.9 % in the wild salmon and all samples from farmed fish were positive. The quantity of PRV infection was variable as demonstrated by the range of detected Ct values in the samples, i.e.19.8-43.9. The ELF1A Ct values of all samples analyzed were within our defined reference conditions.
These results enhance interest in screening samples from various groups of salmon, as well as from other Salmonids in Iceland i.e. Arctic charr, brown trout and rainbow trout.

SUSCEPTIBILITY OF ATLANTIC SALMON (*SALMO SALAR*) TO A NEW VIRUS ASSOCIATED WITH HEART INFLAMMATION AND ANAEMIA IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

H. HAUGE[1]*, T. TAKSDAL[1], Ø. WESSEL[2], A.-G. GJEVRE[1], M.J. HJORTAAS[1], A.B. OLSEN[1], M. DAHLE[1]

[1]*The Norwegian Veterinary Institute*
[2]*Norwegian University of Life Sciences, Oslo, Norway*

From August 2013 to January 2014 the Norwegian Veterinary Institute (NVI) unraveled the pathology of disease outbreaks in rainbow trout from four hatcheries on the Norwegian west coast. The findings were anaemia, inflammation in heart- and red muscle and liver necrosis. Mortalities varied from low to high (21%) in affected tanks. A sequence from an RNA virus resembling piscine orthoreovirus (PRV) has been detected in diseased rainbow trout from all four hatcheries and in contact sites to these hatcheries, including brood fish and fish transferred to seawater sites. Although this indicates that the virus could be associated with the disease this has not yet been confirmed. Neither has virus cultivation in cell cultures been successful so far. NVI conducted a pilot infection trial in 2014 to investigate if the disease is transmissible. This small scale study showed that the amount of the PRV-related virus increased in the blood of rainbow trout injected with infected material. The virus was also transferred to cohabitant fish. Clinical symptoms and mortality were not observed during the trial period of eight weeks, but histopathological findings in one fish could indicate that the disease was transferred. The infection trial also showed that the amount of virus increased in the blood of Atlantic salmon injected with infected material. However, the virus level increased slower compared to rainbow trout, and transfer to cohabitants was not seen within the time frame of the pilot study. If Atlantic salmon is susceptible to the new PRV-related virus and develops disease, this could have serious consequences to the salmon farming industry. We have therefore conducted a controlled long term infection trial to answer this question. Results from the pilot and main trial will be presented.

PISCIRICKETTSIA SALMONIS INFECTION IN EUROPEAN SEA BASS – AN EMERGING DISEASE IN MEDITERRANEAN MARICULTURE

S. ZRNČIĆ[1]*, N. VENDRAMIN[2], T.S. BOUTRUP[3], M. BOYE[2], M.S. BRUUN[2], D. BRNIĆ[1], D. ORAIĆ[1]

[1]*Croatian Veterinary Institute*
[2]*Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark*
[3]*Aquapri Innovation*

During late autumn 2013, two marine fish farms located in Croatia experienced disease outbreak leading to 20% mortality in the affected batch. Clinical disease appeared when water temperature was below16^{o}C. Affected European Sea bass (*D. labrax*), weighting approximately 30 grams, showed abnormal swimming behavior, mainly characterized by whirling movements. Fish stop feeding and finally died few days after the appearance of clinical signs. Diseased specimens displayed pale livers, empty digestive tract with distended gall bladder; the examination of the brain displayed hyperemia and congestion of the meninges. Bacteriological examination performed seeding heart, kidney and spleen on several bacteriological media incubated at 22^{o}C during 48 hours did not show growth of any bacteria. Virological examination of brain homogenate, performed to rule out Nodavirus infection, using both susceptible cell culture SSN-1 and RT-qPCR tested negative. Histological examination of the brain tissue revealed multi focal areas of necrosis and extensive inflammation with predominantly histiocytic cells. Inflammatory cells displayed intra-cytoplasmatic basophilic vacuoles. The lumen of the brain ventricul appeared to be filled with cellular debris and abundant presence of eosinophilic macrophages was recorded. Based on these findings the suspicion of *Piscirickettsia salmonis* infection was formulated. Fluorescent in situ hybridization (FISH) using an oligonucleotide probe generically targeting the 16S rRNA region of bacteria tested positive once applied to fixed tissue sections of affected fish. The pathogen was identified by 16S rRNA gene sequencing using whole frozen brain material from affected fish and the sequence showed 99% similarity with *P.salmonis*. Finally, from the sequencing results a FISH probe for *P.salmonis* were designed and applied to histology section from affected fish, and this showed specific binding to the bacteria observed in the brain tissue sections.
This pathogen has mainly been associated with disease in Atlantic salmon, and few reports in the Mediterranean basin are available. Further analysis will be attempted characterize it more precisely.

EPITHELIOCYSTIS IN FARMED ORANGE-SPOTTED GROUPER (*EPINEPHELUS COIOIDES*)

A. TAYLOR-BROWN[1], A. BRIDLE[2], T. MILLER[3] B. NOWAK[2]*, P. TIMMS[1], A. POLKINGHORNE[1]

[1]*University of Sunshine Coast, Sippy Downs, Australia*
[2]*University of Tasmania, Launceston, Australia*
[3]*James Cook University, Cairns, Australia*

Epitheliocystis is a common condition caused by intracellular infection with bacteria from Chlamydiales of epithelium of gills or skin of fish. So far the pathogen appears to be host-specific. Orange-spotted grouper is an emerging tropical aquaculture species from the *Epinephelinae* subfamily of Serranids. Typical epitheliocystis pathology, including some gross pathology was initially observed in a few individuals examined for gill flukes. This resulted in sampling of cohorts from a number of farms in northern Queensland followed by histological analysis. Round to oblong basophilic inclusions ranging from 10 μm to 100 μm in size were present in the gills. Cysts were sporadically spread throughout the filaments ranging from 0 to 35 cyst/filament. All cysts exhibited the characteristic granular appearance. While most cysts were enclosed in hyaline capsules, some cysts were walled off by eosinophilic capsules; presumably layers of epithelial cells. There was a large variation in hyperplastic response. Gill samples were screened for the presence of chlamydial DNA using pan-*Chlamydiales* primers. PCRs covering the near full length 16S rRNA gene were then employed for a subset of samples, and sequencing of ~1400 bp amplicons confirmed these *Chlamydiae* to be a novel species in the Ca. *Parilichlamydiaceae* family. The pathogen's presence was confirmed in the infected fish using molecular methods (PCR and ISH).

KEY VIRULENCE FACTORS OF *PHOTOBACTERIUM DAMSELAE* SUBSP *DAMSELAE* AND SUBSP *PISCICIDA* ARE ENCODED WITHIN MOBILE GENETIC ELEMENTS

C.R. OSORIO*, M. BALADO, A.J. RIVAS, B. PUENTES, A. VENCES, M.L. LEMOS

Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Photobacterium damselae is a member of the *Vibrionaceae* family that includes two subspecies, subsp. *piscicida* (PDP) and subsp. *damselae* (PDD) which are closely related, although they show clear phenotypic differences. PDP is the causative agent of marine fish pasteurellosis, while PDD causes vibriosis in a variety of fish and other marine animals. A number of key virulence factors have been identified in the two subspecies. Although some of these factors are unique for each subspecies, they have in common that they are encoded within plasmids and other mobile genetic elements. One of the main virulence factors in PDD is the production of several hemolysins, two of which ($HlyA_{pl}$ and Dly) are encoded within the 153 kb conjugative plasmid pPHDD1. The third one ($HlyA_{ch}$), is encoded within a hyper-variable chromosomal region that contains features related to mobile elements. These hemolysins are not present in any PDP strains. However, PDP synthesizes a potent apoptotic toxin (AIP56), which is also encoded within a plasmid (pPHDP10). Both subspecies possess diverse siderophore-based mechanisms for iron assimilation within the host. We have demonstrated that European strains of PDP obtain iron by the synthesis of siderophore piscibactin. The synthesis and transport of this siderophore are encoded within a plasmid of 69 kb (pPHDP70) which is a key virulence factor for strains harbouring it, and can be mobilized to and expressed in other bacteria. The piscibactin genes constitute a pathogenicity island with high similarity to the yersiniabactin siderophore-encoding HPI of *Yersinia*. Piscibactin production was never detected in PDD. In contrast, some PDD strains harbour a complete gene cluster for the synthesis and transport of vibrioferrin, a siderophore previously described in other vibrios. Again, this gene cluster is part of a pathogenicity island that seems to have spread among different bacteria by horizontal gene transfer. We have also described in PDP strains a third plasmid (pPHDP60) that encodes a type II secretion system with a yet undefined role in virulence. This work highlights the diversity of the virulence factors of the two subspecies of *P. damselae* and their codification by mobile elements.

TAIL AND FIN ROT BACTERIA IN WILD AND FARMED FISH IN ICELAND. STRAIN DIVERSITY AND INFECTION ROUTE

S. HJARTARDOTTIR*, G. GUTTORMSDOTTIR, T. EINARSDOTTIR

Institute for Experimental Pathology, University of Iceland Keldur, Reykjavik

Infections caused by fin and tail rot bacteria are an increasing problem in fish farming in Iceland as well as worldwide. In order to estimate the diversity of these bacteria in Iceland, fin and tail rot bacteria were isolated from ulcers of several different marine and freshwater fish species, wild and captive; fish size ranging from small fingerlings to mature individuals. Samples for examination were furthermore obtained from a hatchery station, both fertilized and unfertilized roe, and from intake water supplies of fish farms. The variability of bacterial strains was analyzed within and between fish species and environment and the possibility of a vertical infection route was estimated.

Swabs were taken from fin ad tail ulcers and either inoculated directly on Flexibacter Maritimum Medium (FMM) agar or first diluted with sterile Marine Salt Solution (MSS). Roe were washed with sterile water, mashed in a stomacher bag and then streaked on FMM agar. Samples from intake water were filtered through bacterial tight filter and streaked on FMM agar.

DNA was extracted from strains representing phenotypic characteristics of tail and fin rot bacteria and their 16S rRNA gene sequenced. Altogether, nearly four hundred strains were isolated. Sequencing of the 16S rRNA gene indicate strain relationship between stations. Multilocus sequence typing (MSLT) for *F. psychrophilum* strains is currently being set up using the trpB, gyrB, dnaK, fumC, murG, tuf and atpA genes, in order to analyze the relationship between these strains.

Our results indicate that there is a large strain variance between tail and fin rot bacteria in Iceland and a possible host specificity of some strains. We hope that further analysis of *F. psychrophilum* by MLST will help us to estimate the transmission route of the bacteria in freshwater farming.

INVESTIGATION ON *TETRACAPSULOIDES BRYOSALMONAE* LIFE-CYCLE IN BRYOZOAN *FREDERICELLA SULTANA* FOLLOWING THE FIST PKD OUTBREAK IN AUSTRIA

B. GORGOGLIONE[1]*, M. KOTOB[1,2], G. UNFER[3], M. EL-MATBOULI[1]

[1]*Clinical Division of Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, Vienna, Austria*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt*
[3]*Institute of Hydrobiology and Aquatic Ecosystem Management, BOKU University of Natural Resources and Life Sciences, Vienna, Austria*

Myxozoan parasites have never before caused major issues for Austrian wild and farmed fish populations. During the summer 2014, following mortality outbreaks happening in the context of a syndrome locally so-called "Black Trout", was diagnosed Proliferative Kidney Disease (PKD) for the first time in Austria. A farm pre-restocking small scale test confirmed the infection with *Tetracapsuloides bryosalmonae* in juvenile autoctone brown trout (*Salmo trutta*). Bryozoan *Fredericella sultana* colonies were retrieved from several locations along the same river, up to 6 km upstream the infected farm. Large branching colonies were extensively encrusting the iron pipelines in the farm receiving unfiltered water from the river Kamp, a tributary of the Danube. Submerged dead pieces of wood and cobblestones acted as substrate for bryozoans in turbulent segments of little side-creeks to the main river course. *T. bryosalmonae* 18S ribosomal DNA amplification was achieved with a TaqMan PCR approach. Overt *T. bryosalmonae* infection was seen in *F. sultana* zooids with large mature spore sacs releasing infective malacospores. A rich eco-system was observed, with an assortment of invertebrates association with bryozoan colonies, including free-living ciliates, bdelloid rotifers and several species of tubuficid oligochaetes. Commensal ectosymbionts, including sessiline peritrichs, *e.g. Stentor* sp. and *Vorticella* sp., used chitinous walls of viable bryozoan zooids as substrate to firmly attach their stalk to. Bryozoan colonies were adapted to laboratory conditions, with water temperatures set to 17 °C, simulating the summer season hypertrophycation and thus allowing faster growing for bryozoans and commensals organisms. A gradual increase of water temperatures has been recorded over the recent years in river Kamp. A changing environment, with improved trophic conditions and more favourable water temperatures for the development of overt infections in bryozoan, could explain this fist PKD outbreak onset in Lower Austria. Further research is needed to assess the *T. bryosalmonae* life cycle interactions and disease incidence in Austria as around the river Danube basin.

PANCREAS DISEASE – WILL THE DIFFERENT PD SITUATION IN THE SOUTH AND MID PART OF NORWAY CONTRIBUTE TO MAINTAIN THE CHALLENGE PRESSURE AND FURTHER SPREAD OF THE DISEASE?

T. HOVLAND*, L. MELSTVEIT

MSD Animal Health, Bergen Norway

Management of PD in Norway is not uniform despite the fact that the disease is well documented as highly contagious, and that the virus is effectively spread from farm to farm.
Several measures have been implemented from the industry. These have contributed positively to reduce the infection pressure, and the severity of the disease in certain areas.
However, PD continues to spread further north. In the mid part of Norway the disease is now endemic only a few years after the first outbreak was registered.
In 2014 most of the official PD detections were registered in the counties Hordaland, Sør Trøndelag and Rogaland. These are the counties holding the highest density of fish farms in Norway.
The results from monitoring PD in Norway for some years shows that both salmon and rainbow trout are sea transferred into areas with already PD infected fish from different fish generations present. In addition, the results also show that high density of marine sites in some areas also generates increased wellboat traffic between the sites to and from the harvesting plants.
Updates on the PD situation in the different areas of Norway will be presented, and accompanying risk factors will be discussed.
The different handling of PD along the Norwegian coast and the fact that the spread of the disease seems to proceed indicates that the present legislation should be evaluated and revised, leading to a more uniform management of this contagious and economically important virus disease for the fish farming industry.

EFFECT OF PD-VACCINATION ON SHEDDING OF SPDV POST CHALLENGE

K. ULVEN*, I. SOMMERSET, P. SKJOLD, A. TANGERÅS, P. FROST, S. VILLOING

MSD Animal Health, Bergen Norway

Epidemiological studies of PD outbreaks as well as laboratory data have shown that SPDV has a high potential for water-borne spreading following viral shedding from infected fish.
Following experimental challenges SPDV is detected in mucus and faeces collected from infected fish. In addition, infective virus is also sampled from the water.
The peak of SPDV shedding correlates with the peak of virus in the blood (viremia), which happens at an early time point in the infection. Under field conditions, release of infective SPDV in the water may also occur from diseased/dead fish being subjected to degradation.
In order to evaluate whether PD-vaccination could reduce shedding of SPDV in an infected population, vaccination-challenge studies were conducted. Data collected at different time intervals post challenge, will be presented.
Results from blood and faeces, as well as water samples showed that vaccinated fish exposed to SPDV had a significantly lower prevalence of individuals shedding virus than non-vaccinated fish.
Reduction of spread of highly contagious viruses is the principle of herd immunity obtained by vaccination of the entire population of hosts and applies to both humans and fish.

THE STRESS HORMONE CORTISOL DIRECTLY IMPACTS *FLAVOBACTERIUM COLUMNARE* IN VITRO GROWTH CHARACTERISTICS

A.M. DECLERCQ*, J. AERTS, B. AMPE, F. HAESEBROUCK, S. DE SAEGER, A. DECOSTERE

Ghent University, Ghent, Belgium
Institute for Agriculture and Fisheries Research, Melle, Belgium

For the majority of bacterial fish pathogens, the role of stressors is considered a key factor in disease outbreaks. Based upon studies in mammals, there is considerable evidence to suggest that, besides eliciting an impairment of the immune system, stress hormones can have a direct effect on bacterial cells. Hitherto, this intriguing field of "microbial endocrinology", whereby micro-organisms, through their long co-existence with animals, have developed sensory systems for detecting host-associated hormones, has remained largely unexplored in aquatic diseases. In this respect, the purpose of the present study was to investigate the *in vitro* impact of the stress hormone cortisol on *Flavobacterium columnare*, the causative agent of columnaris disease, a much-feared and predominant bacteriosis of freshwater fish. To do so, *F. columnare* isolates of different virulence were cultivated in the presence of cortisol and the impact on bacterial titres and colony morphology assessed.
Exposure of the highly virulent isolates to cortisol resulted in decreased bacterial titres. In addition, the retrieved colonies were smaller and displayed a significantly less rhizoid appearance and spreading as exhibited by shorter radiating tendrils compared to colonies procured from unsupplemented broth. For the low virulent isolates, no significant differences were noted whether or not the growth medium was supplied with cortisol.
These results are particularly interesting as rhizoid colonies with spreading edges reflect the gliding motility of the harboured *F. columnare* cells. This gliding trait is lost as bacteria become immobile and start forming microcolonies on the fish's skin and gills, as a first step in biofilm formation, which is a well-known feature in fish succumbing to columnaris disease. This urges us to speculate that fish harbouring high cortisol levels in the skin and gill mucus, are more prone to microcolony and consequent biofilm formation by highly virulent *F. columnare* isolates. This may explain the individual variation in terms of disease susceptibility as the magnitude of mucus cortisol levels may differ markedly in between fish following stress stimuli.
This study is the first to demonstrate a direct effect of cortisol on a fish pathogenic bacterium and hence engenders a new perspective to bacteria-host communications in aquaculture.

EFFECT OF TEMPERATURE AND DIET ON WOUND HEALING IN ALTANTIC SALMON (*SALMO SALAR* L.)

L.B. JENSEN[1]*, T.WAHLI[2], C. MCGURK[1], T.B. ERIKSEN[1], A. OBACH, R. WAAGBØ[3], A. HANDLER[4], C. TAFALLA[4]

[1]*Skretting Aquaculture Research Centre, Stavanger, Norway*
[2]*Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[3]*National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway*
[4]*Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain*

Compromised skin integrity of farmed Atlantic salmon, commonly occurring under low temperature and stressful conditions has major impacts on animal welfare and economic productivity. Even fish with minimal scale loss and minor wounds can suffer from secondary infections, causing downgrading and mortalities. Wound healing is a complex process, where water temperature and nutrition play key roles. In this study, Atlantic salmon (260 g) were held at different water temperatures (4 or 12°C) and fed three different diets for 10 weeks, before artificial wounds were inflicted and the wound healing process monitored for two weeks. The fish were fed either a control diet, a diet supplemented with zinc (Zn) or a diet containing a combination of functional ingredients in addition to Zn. The effect of diet was assessed through subjective and quantitative skin histology and the transcription of skin-associated chemokines. Histology confirmed that wound healing was faster at 12°C. The epidermis was more organized, and image analysis of digitised skin slides showed that fish fed diets with added Zn had a significantly larger area of the epidermis covered by mucous cells in the deeper layers after two weeks, representing more advanced healing progression. Constitutive levels of the newly described chemokines, herein named CK 11A, B and C, confirmed their preferential expression in skin compared to other tissues. Contrasting modulation profiles at 4ºC and 12ºC were seen for all three chemokines during the wound healing time course, while the Zn-supplemented diets significantly increased the expression of CK 11 A and B during the first 24 hours of the healing phase.

DEVELOPMENT OF AN EVALUATION SCORE FOR EXAMINATION AND COMPARISON OF METHODS AND PROCEDURES FOR STUNNING AND SLAUGHTERING OF RAINBOW TROUT (*ONCORYNCHUS MYKISS*) AND CARP (*CYPRINUS CARPIO*)

V. JUNG-SCHROERS*, J. HELLMANN, K. RETTER, D. STEINHAGEN

Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany

In Germany stunning of animals is regulated in a directive. Stunning of fish in general is allowed due to percussion or electric current. Additionally, only for salmonids stunning by using CO_2 in a water bath is possible. There are no precise instructions on how these methods should be used.
In this study the whole process of stunning and slaughtering of carp and rainbow trout was evaluated. This process includes catching fish from the ponds, keeping fish in special tanks before slaughter, transport of fish from these tanks to the stunning site and finally stunning and killing of fish. This process was evaluated in 19 aquaculture farms which were slaughtering trout and 12 farms which were slaughtering carp. Trout were stunned by electric current in 8 farms, by percussion in 8 farms, by electric current combined with percussion in 2 farms and by CO_2 in 1 farm. In 6 farms carp were stunned by electric current, in 3 farms by percussion and 5 farms used a combination of electric current and percussion. The whole process was recorded in all facilities and samples from water of transport tanks, keeping tanks and stunning tanks were analyzed for important parameters like oxygen, pH, temperature, ammonia, nitrite, organic content and cortisol. Blood was taken from 6-10 fish per facility or treatment and parameters like cortisol, hematocrit, glucose, lactate and different ions were measured.
An evaluation score with 93 points was established which includes all measured parameters and data about the process. Different evaluation factors were multiplied with scores assessing their importance. For example, very important factors like the number of fish which were not stunned before slaughter got the highest multiplication scores. An overall evaluation score was calculated, combining the scores from different aspects of the harvesting process, such as keeping, transportation, stunning and killing.
With the overall evaluation score an assessment and a gradual classification of different techniques and methods for stunning and killing of fish is possible. Critical procedures or methods can be detected. Therefore the overall evaluation score can also lead to recommendations like electric parameters or stunning time for electrical stunning.

GENOME SEQUENCING OF FOUR STRAINS OF *EDWARDSIELLA ICTALURI* FROM THE MEKONG DELTA REVEALS HIGH SIMILARITY TO MEGAPLASMIDS PREVIOUSLY DESCRIBED IN HUMAN PATHOGENS

A. KLEVAN[1]*, T.T. VO[2], P.T. NGUYEN[2], P.D. TRAN[2], T.H.T. NGUYEN[1], A. NYGAARD[1], M. BORDEVIK[1], K. GRAVNINGEN[1]

[1]*PHARMAQ AS, Oslo, Norway*
[2]*PHARMAQ Vietnam Ltd., Ho Chi Minh City, Vietnam*

Edwardsiella ictaluri, the causative agent of enteric septicemia of catfish (ESC), is a well known bacterial pathogen with significant economical losses in both Vietnamese and American aquaculture. Antibiotics are used for treatment and there has been identified resistance towards a large range of antibiotics, which causes concern to the industry, the environment and human health. To better understand if there are important variations among different isolates of *E. ictaluri* from the Mekong Delta, we have analyzed several isolates from diseased farmed striped catfish (*Pangasianodon hypophthalmus*). Although these strains display some variation in terms of virulence and plasmid content, they are indistinguishable by phylogenetic analysis of the 16S, *gyrB* and *glnA* genes. Therefore, to fully uncover any differences, we have performed full genome sequencing using Illumina technology on four different isolates. Preliminary results reveal that isolates from the Mekong Delta are significantly different from the American *Edwardsiella ictaluri* 93-146 strain. Furthermore, our comparative analyses show that the four strains are strikingly similar to each other, being almost identical at the genomic level. However, we have identified sequences longer than 100 kb with high DNA identity to megaplasmids previously described in human pathogens. These findings strengthen the concerns related to transfer of antibiotic resistance between aquaculture and the general community.

HISTOPATHOLOGICAL EVIDENCE OF MYCOBACTERIOSIS IN MULLETS FROM SARDINIAN LAGOONS (CENTRAL-WESTERN MEDITERRANEAN)

E. ANTUOFERMO[1, 2]*, R. ARIU[1], M. POLINAS[1], A. PAIS[2], T. CUBEDDU[1], M.A. SANNA[1], V. VITIELLO[1], M. PREARO[3]

[1]*Department of Veterinary Medicine, University of Sassari, Sassari, Italy*
[2]*Laboratory of Aquaculture and Aquatic Resources Management, Section of Animal Sciences, Department of Agriculture, University of Sassari, Sassari, Italy*
[3]*State Veterinary Institute of Piedmont, Liguria and Aosta Valley, Fish Disease Laboratory, Torino, Italy*

Fish mycobacteriosis is a chronic progressive disease caused by nontuberculous (atypical) mycobacteria. It can cause low mortality in wild aquatic organisms but significant financial loss in farmed fish. The disease may be detected in fish without clinical symptoms and can be also pathogenic for humans. Its diagnosis is mainly based on histopathological evidence of granulomas and acid-fast bacteria revealed by Ziehl-Neelsen (ZN) stain. Since mycobacteriosis in the Mugilidae family has been scarcely reported worldwide (and in particular in the Mediterranean Sea), the aim of this work was to find out its occurrence in extensively reared mullets from 4 different coastal lagoons of Sardinia (Italy). This was carried out by providing a histopathological description of granulomas at different stages of evolution.
Two hundred thirty-nine mullets were sampled in late summer 2014 and a complete necropsy was performed. Samples of heart, liver, spleen and kidney were formalin fixed, paraffin embedded and stained with Hematoxylin and Eosin, ZN and Masson's trichrome stains. Small whitish nodules were macroscopically observed in spleen and 8 out of 239 specimens showed positive ZN stain granulomas for mycobacteria. Based on histological pattern, granulomas were classified in 3 categories associated to their evolutive stage: 1) early stage characterized by macrophage aggregates without necrosis; 2) intermediate stage composed of macrophages with a central core of coagulative necrosis; and 3) late stage with a central lytic necrosis delimitated by several layers of fibroblasts but without macrophages. Occasionally, this latter presented necrotic material arranged in a lamellar concentric pattern. Intermediate and late granulomas were the most frequently stages found and acid-fast bacteria were detected in each granuloma stage. Our observations demonstrate for the first time the occurrence of mycobacteriosis in extensively reared mullets from Sardinia. Furthermore, the results achieved evidence that granulomas are mainly found at different stages in the spleen of mullets, confirming the systemic nature of the disease.

RISK FACTOR ANALYSIS OF HUMAN INFECTION WITH *ANISAKIS* SPP. IN THE EUROPEAN ANCHOVY AND SARDINE FROM THE EASTERN ADRIATIC SEA

I. BUŠELIĆ[1]*, J. HRABAR[1], A. ČALIĆ[1], I. RADONIĆ[1], V. POLJAK[2], I. MLADINEO[1]

[1]*Institute of Oceanography and Fisheries, Split, Croatia*
[2]*Croatian National Institute of Public Health, Zagreb, Croatia*

The consumption of thermally unprocessed or lightly processed traditional seafood represents a risk of anisakiasis, considered one of the most significant fish-borne parasitic infections in humans today. The disease is caused by ingestion of live third stage *Anisakis* spp. larvae present in parasitized fish or cephalopods. Thermally unprocessed or lightly processed anchovies (*Engraulis encrasicolus*) and sardines (*Sardina pilchardus*) are basic ingredients of numerous traditional Mediterranean dishes. Therefore, our objective was to genetically identify *Anisakis* spp. and determine its prevalence and intensity in the European anchovies (N= 785) and sardines (N=789) collected during 2 years from the eastern Adriatic Sea. The UV-Press method was used for visual inspection of flattened, deep-frozen fillets and viscera as it conveniently utilises fluorescence of frozen anisakids. A subsample of isolated larvae was identified to species level using mitochondrial marker cytochrome oxidase 2 (CO2). Both larvae isolated from anchovy and sardine confirmed their clustering within *Anisakis pegreffii* sister group, as usually recorded in the Adriatic Sea. The overall prevalence in the European anchovy was 29.70% (95% CI 26.56–32.98), mean abundance 0.71 (bootstrap 95% CI 0.60–0.84) and mean intensity 2.41 (bootstrap 95% CI 2.13–2.73) in contrast to 2.50% (95% CI 1.63–3.90) overall prevalence, 0.03 (bootstrap 95% CI 0.02–0.05) mean abundance and 1.30 (bootstrap 95% CI 1.05–1.60) mean intensity in sardine. Prevalence in fillets was 3.2% (95% CI 2.15–4.69) in anchovy and 0.5% (95% CI 0.18–1.3) in sardine. Subsequently, collected epidemiological data were used to perform risk factor analysis of human *Anisakis* spp. infection, indicating a greater risk of consumption of unprocessed European anchovies than sardines.

The study has been financed by projects FP7 PARASITE (Parasite risk assesment with integrated tools in EU fish production value chains, GA# 312068), Croatian National Scientific Fund HRZZ Angel (GA# 5576) and European Social Fund GA# HR.3.2.01-0276.

ANISAKIS SPP. FROM LARGE PELAGIC FISH OFF EASTERN ADRIATIC - MOLECULAR IDENTIFICATION AND RISK ANALYSIS

J. HRABAR[1]*, I. BUŠELIĆ[1], I. RADONIĆ[1], V. POLJAK[2], I. MLADINEO[1]

[1]*Institute of Oceanography and Fisheries, Split, Croatia*
[2]*Croatian National Institute of Public Health, Zagreb, Croatia*

Nematodes of the genus *Anisakis* Dujardin, 1845 are considered an emerging public health issue due to anisakiasis, a zoonotic disease caused by consumption of raw or lightly processed infected seafood. Previous study conducted in county of Dalmatia, Croatia, has shown particularly high anti-*Anisakis* IgE seroprevalence among island population where thermally unprocessed, salted or marinated fish is a staple food.
In order to assess occurrence of *Anisakis* larvae in fish frequently consumed among Dalmatian island population and possible health risks, we concentrated in collecting large and valuable four pelagic fish species, caught off the coast of Dalmatian islands (chub macekerel, *Scomber japonicus*, horse mackerel, *Trachurus trachurus*, little tunny, *Euthynnus alletteratus* and atlantic bluefin tuna, *Thunnus thynnus*). Fish were eviscerated, filleted into butterfly fillets, pressed under hydraulic press and examined under UV light to reveal the presence of larvae in visceral mass and fillets. Prevalence, mean intensity and mean abundance were calculated using Quantitative Parasitology 3.0 software. For molecular identification genomic DNA was isolated. A ~650 bp fragment of mitochondrial cytochrome oxidase 2 (COX2) locus was amplified and sequenced. Obtained sequences were aligned and a phylogenetic tree has been inferred using Bayesian Inference.
In total 349 *Anisakis* larvae were isolated, while total prevalence was 64,4% (Sterne's exact 95% CI 0,48 to 0,77), mean intensity 12,03 (bootstrap 95% CI 7,03 to 20,55) and mean abundance 7,76 (boostrap 95% CI 4,16 to 13,67). All three values differ between species, with highest prevalence observed in little tunny (*E. alletteratus*), while highest mean intensity and mean abundance were observed in chub mackerel (*S. japonicus*). Molecular identification revealed *A. pegreffii* to be the predominant species, while only few *A. simplex* were identified. Although both *A. pegreffii* and *A. simplex* are capable of penetrating into fish muscle, the later has up to 12 times higher ability to migrate *postmortem* into fillets. Given this fact and epidemiological data collected, the risk of infection by consumption of targeted fish among Dalmatian island population is moderate.

The study has been financed by projects FP7 PARASITE (Parasite risk assesment with integrated tools in EU fish production value chains, GA# 312068), Croatian National Scientific Fund HRZZ Angel (GA# 5576) and European Social Fund GA# HR.3.2.01-0276.

IS STRESS IN FARMED SALMON (*SALMO SALAR*) AN IMPORTANT FACTOR FOR SAPROLEGNIOSIS?

M.J. BECKMANN[1]*, C.J. SECOMBES[2], P. VAN WEST[1]

[1]*Oomycete Laboratory, University of Aberdeen, Aberdeen, UK*
[2]*Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, UK*

Saprolegniosis is a major concern to fish farms worldwide, leading to heavy losses due to infection by a filamentous oomycete pathogen. *Saprolegnia* infections are often observed on fish farms after procedures that involve handling or movement of the fish, which most likely induce significant stress to the fish. The routine vaccination of salmon pre-smolts against viral and bacterial diseases is the cause for increased susceptibility to the oomycete pathogen *Saprolegnia parasitica.* Saprolegniosis is observed approximately 8-12 days after the intraperitoneal vaccination if fish are not being treated preventatively during the following two weeks after the procedure. This study presents for the first time stress and immune gene expression profiles over the first 9 days after vaccination. We aimed to distinguish between two key factors of the vaccination procedure which are (1) stress, due to the physical processing of the fish and (2) the immune modulatory effect of the vaccine itself. The blood parameters cortisol and glucose clearly demonstrate the induction of a strong stress response due to the vaccination procedure which is more sustained by the injection with vaccine. We demonstrate the initiation of a strong pro-inflammatory/antimicrobial response in vaccinated fish while the vaccination procedure itself causes some degree of down-regulated gene activity. Stress gene expression for the glucocorticoid receptor, heatshock protein 90 and 70, steroidogenic acute regulatory protein acute regulatory protein (StAR) and Cytochrome P450 side-chain-cleavage (P450scc) show also differential regulation in response to the vaccination practice.

PATHOGEN-SPECIFIC IMMUNOGLOBULIN AND B CELL RESPONSES IN THE GILLS OF WILD-TYPE AND IGT$^+$ B CELL-DEPLETED RAINBOW TROUT

Z. XU[1], D. PARRA[1], F. TAKIZAWA[1], D. GÓMEZ[1], L. VON GERSDORFF JØRGENSEN[2], S. LAPATRA[3], J.O. SUNYER[1]*

[1]*School of Veterinary Medicine, Department of Pathobiology, University of Pennsylvania, Philadelphia, PA, 19104, USA*
[2]*Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-1870 Frederiskberg C, Denmark*
[3]*Research Division, Clear Springs Foods Inc., Buhl, ID 83316, USA*

We have previously demonstrated that IgT is an immunoglobulin specialized in gut and skin mucosal immunity. Thus, we hypothesized that IgT might play a pivotal role in gill mucosal immunity. However, it has recently been suggested a role for IgD and putative IgD$^+$/IgM$^-$ B cells in rainbow trout gill immunity. Hence, the main goal of this study was to assess which immunoglobulin and B cell responses were induced in the gill of rainbow trout upon pathogenic challenge. Here we show that IgT$^+$ B cells represent the major B cell subset in the gill filaments. In contrast to reported results by others, we found that all gill B cells expressing surface IgM, also expressed surface IgD and, that the percentage of B cells solely expressing either IgD or IgM was negligible. More importantly, significant specific-IgT immune responses against *Ichthyophthirius multifiliis* (Ich) were measured in the gill mucus, while IgM responses were almost exclusively detected in the serum. In contrast, Ich-specific IgD was absent both in gill mucus and serum. While these data points to a pivotal role of IgT in teleost gill mucosal immunity, we asked whether IgT was required for pathogen clearance. To address this critical question we developed a unique IgT B-cell depletion trout model. Upon depletion treatment, IgT$^+$ B cells from gills were depleted by over 95% for a 7 week period. In contrast the % of IgM$^+$ B cells did not change. Upon IgT$^+$ B-cell depletion, fish were sublethally challenged with Ich. After two weeks post-challenge, a significant percentage of mortality occurred in the IgT B-cell depleted groups (25-50%). Critically, pathogen load was dramatically higher in the IgT B-cell depleted groups when compared to control fish. Interestingly we could never observe IgM or IgD compensatory responses against Ich. In conclusion, these data shows that IgT is the main immunoglobulin player in gill mucosal immunity and, that IgT is essential for pathogen control and clearance. Our results have critical implications for the future design of fish vaccines and immunostimulants that induce gill mucosal immunity.

DIFFERENTIAL HUMORAL IMMUNE RESPONSE OF GILTHEAD SEA BREAM (*SPARUS AURATA*) RESISTANT TO *ENTEROMYXUM LEEI* (MYXOZOA) RE-INFECTION

I. ESTENSORO, R. DEL POZO, A. SITJÀ-BOBADILLA*

Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), Castellón, Spain

Enteromyxosis in gilthead sea bream (GSB) provokes severe chronic enteritis leading to emaciation, caquexia and high mortalities. This study focuses on the humoral immune response of GSB, by the detection of the specific circulating antibodies and the measurement of serum peroxidases. Survivor GSB from previous *E. leei* experimental infections (n = 21) and naïve GSB (n = 20) were equally distributed into two tanks and exposed to *E. leei*-contaminated water effluent from a donor tank during 175 days. Blood samples were taken at 0, 66, 105 and 175 days post exposure (dpe) from both groups and sera obtained. Parasite diagnosis was performed by non-lethal PCR at 0, 66, 105 dpe (rectal probe) or by PCR and histology (posterior intestine) at 175 dpe. Specific antibodies against *E. leei* (Ab-Eleei) were immunohistochemically detected on infected intestinal GSB tissues by their immunoreactivity against the parasite. Total serum IgM was detected by means of ELISA with a Pab against GSB IgM. Total serum peroxidases were measured in a plate assay by incubating with 3,3V,5,5V-tetramethylbenzidine hydrochloride.

Prevalence of infection in survivor fish was 5.8 % (0 dpe), 7.8 % (66 dpe) and then dropped to 0, whereas in naïve fish it reached 40 % at 105 dpe and then lowered to 22 % at 175 dpe. The percentage of survivor fish with Ab-Eleei was similar throughout the experiment (67-76 %), whereas naïve fish lacked detectable Ab-Eleei until 105 dpe (only 20 % fish had specific antibodies), reaching 67 % at 175 dpe. Similarly, total serum IgM was significantly higher in survivor than in naïve fish at 0 and 175 dpe, but it peaked later in survivor (175 dpe) than in naïve (105 dpe) fish. Peroxidases were significantly higher in naïve fish in all sampling points, except at 175 dpe, when they dropped to the levels found in survivor animals.

Therefore, survivor GSB have acquired immunity and resistance to the re-infection, which seems to be related to Ab-Eleei levels, as survivor had higher percentage of Ab-Eleei^{+} fish with higher IgM levels from the beginning, whereas in naïve fish the acquirement of Ab-Eleei took more than 3 months. By contrast, naïve fish had a higher level of peroxidases, which seemed "switched off" in survivor fish.

This work was funded by Spanish MINECO through projects AGL2009-13282 and AGL2013-48560 and partially supported by the "Generalitat Valenciana" (projects PROMETEOII/2014/085 and ISIC 2012/003).

A MULTICOLOUR FLOW CYTOMETRY FOR THE SIMULTANEOUS ANALYSIS OF RESTING AND PROLIFERATIVE B AND T LYMPHOCYTE RESPONSES FOLLOWING PATHOGENIC CHALLENGE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS)*

T. KORYTÁŘ*, F. TAKIZAWA, Z. XU, J.O. SUNYER

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

The attempts to investigate cellular immune responses in fish species has been hampered by the absence of reliable monoclonal antibodies recognizing different fish leukocytes populations. In the past we have produced mAbs to rainbow trout IgT and, more recently to rainbow trout CD4, which have enabled the characterization of IgT^+ B cell and $CD4^+$ T cell subsets respectively. The ability to concurrently detect B and T cell subpopulations offers unprecedented opportunities for the evaluation of the cellular components of the adaptive immune system upon challenge of fish with a pathogen or treatment with a vaccine.

To demonstrate the potential of concomitantly evaluating B and T cell responses in fish, we developed a multicolor flow cytometry method that enabled the simultaneous detection of IgT^+ B cells, IgM^+ B cells, CD4-1^+ T cells, CD4-2^+ T cells and $CD4^+$ monocytes/macrophages. This multicolor flow cytometry approach was then used to analyze the kinetics of B and T cell responses in systemic as well as mucosal organs during two weeks, upon challenge of fish with a sublethal dose of *Yersinia ruckeri*. Overall, our preliminary results show that while the proportion of $CD4^+$ T cells in the blood and spleen increased significantly at the later time points, no major changes were seen in the mucosal organs analyzed. On the other hand, the percentage of B cells did not vary significantly in the systemic organs whereas in the mucosal organs, significant increases and decreases of IgM^+ and IgT^+ B cells were observed over time. These data is currently being complemented with the analysis of *in vivo* proliferation of the aforementioned B and T cell populations, thus providing further insight into the initial stages of lymphocyte mobilization and how this correlates with the development of adaptive immune responses. Moreover, this approach is expected to provide predictive kinetic patterns of resting and proliferating B/T lymphocytes that may foretell whether fish is at the early stages of an immune response or pathogen infection. Critically, such patterns may also be predictive of the effectiveness of fish vaccines, thus enabling a more rationale design of future fish vaccine strategies.

GLOBAL 3D IMAGING OF *YERSINIA RUCKERI* BACTERIN UPTAKE IN RAINBOW TROUT FRY

M. OHTANI[1]*, K.R. VILLUMSEN[1], E.O. KOPPANG[2], M.K. RAIDA[1], A.M. BOJESEN[1]

[1]*University of Copenhagen, Frederiksberg, Denmark*
[2]*Norwegian University of Life Sciences, Oslo, Norway*

Yersinia ruckeri is the causative agent of enteric redmouth disease (ERM) in rainbow trout, and the first commercially available fish vaccine was an immersion vaccine against ERM consisting of *Y. ruckeri* bacterin. The ERM immersion vaccine has been successfully used in aquaculture farming of salmonids for more than 35 years. The gills and the gastrointestinal (GI) tract are believed to be the portals of antigen uptake during waterborne vaccination against ERM; however, the actual sites of bacterin uptake are only partly understood. In order to obtain insight into bacterin uptake during waterborne vaccination, optical projection tomography (OPT) together with immunohistochemistry (IHC) was applied to visualize bacterin uptake and processing in whole rainbow trout fry. Visualization by OPT revealed that the bacterin was initially taken up via gill lamellae from within 30 seconds post vaccination. Later, bacterin uptake was detected on other mucosal surfaces such as skin and olfactory bulb from 5 to 30 minutes post vaccination. The GI tract was found to be filled with a complex of bacterin and mucus at 3 hours post vaccination and the bacterin remained in the GI tract for at least 24 hours. Large amounts of bacterin were present in the blood, and an accumulation of bacterin was found in filtering lymphoid organs such as spleen and trunk kidney where the bacterin accumulates 24 hours post vaccination as demonstrated by OPT and IHC. These results suggest that bacterin is taken up via the gill epithelium in the earliest phases of the bath exposure and from the GI tract in the later phase. The bacterin then enters the blood circulatory system, after which it is filtered by spleen and trunk kidney, before finally accumulating in lymphoid organs where adaptive immunity against ERM is likely to develop.

A NOVEL CHALLENGE METHOD WITH *AEROMONAS SALMONICIDA* IN RAINBOW TROUT FOR EVALUATION OF FURUNCULOSIS VACCINES

M.H. MARANA[1]*, J. SKOV[1], J.K. CHETTRI[1], B. KROSSØY[2], I. DALSGAARD[3], P.W. KANIA[1], K. BUCHMANN[1]

[1]*University of Copenhagen, Frederiksberg C, Denmark*
[2]*Vaxxinova Norway AS, Bergen, Norway*
[3]*Technical University of Denmark, Frederiksberg C, Denmark*

Challenge methods used to induce furunculosis in rainbow trout when testing the potency of vaccines may include exposure to *Aeromonas salmonicida* by intraperitoneal injection, cohabitation or bath immersion. Intraperitoneal injection is effective but will not reflect systemic immunity because inflammatory cells at the vaccine injection site may combat injected bacteria fast. The cohabitation and bath immersion methods both mimic the natural infection route but are less effective in inducing the disease.
We have tested a new challenge method mimicking that rainbow trout in fish farms might be infected with *A. salmonicida* through injured epidermis (fin biting) and our new method resembles closely the natural infection route where bacteria gain access to fish through the lesions. In our challenge procedure small skin lesions were made on the upper part of the caudal fin with a multipuncture device containing 10 needles puncturing the upper tail fin after the fish were anaesthetized. A volume of 100 µl of a 48 hour culture of *A. salmonicida* (3.4×10^8 CFU/ml) was layered at the puncture site for 60 seconds where after fish were placed in freshwater for recovery. This technique proved to be efficient in inducing a more natural disease progression in fish and a stable mortality. The method could differentiate efficacies of different vaccines with regard to adjuvant formulations and content of antigen.

ADJUVANT EFFECT OF A FORMULATED CARBOHYDRATE (MSS1) IN VACCINES AGAINST *AEROMONAS HYDROPHILA* IN COMMON CARP (*CYPRINUS CARPIO*)

N. KAREEM[1, 2]*, A. ADAMS[3], M. SKIDMORE[1], S. HARRIS[1], D. HOOLE[1]

[1]*Keele University, Keele, UK*
[2]*University of Sulaimani, Sulaimaniyah, Kurdistan Region*
[3]*Institute of Aquaculture, University of Stirling, Stirling, UK*

One of the most effective methods of preventing disease in aquaculture is the application of vaccines. The development of safe and effective vaccines is extremely desirable for improving fish health and the reduction of other forms of disease control such as the application of antibiotics. An important component of the vaccine is the addition of adjuvants or immunostimulants to increase vaccine efficiency. These natural immunostimulants have been studied for their ability to stimulate the nonspecific immune responses, alone or in combination with vaccine, and their effect appears to be dose dependant and may be short.

In our previous investigation a laboratory formulated carbohydrate (MSS1), was examined for its effects on the immune status in carp. Fish were injected with 5 $mg.kg^{-1}$ of MSS1 showed inducement (up to 7 days post injection) of innate immune parameters i.e. complement pathway (ACH50), lysozyme activity in serum and genes expression (C3 and Lysozyme).

This study has been extended to establish the role of MSS1 as an adjuvant in formalin killed *Aeromonas hydrophila* vaccine in carp. Carp were injected intraperitoneally with: MSS1 5 $mg.kg^{-1}$, formalin killed *Aeromonas hydrophila* vaccine 1×10^{9} $cell.ml^{-1}$, either individually or in combination and PBS as a control. The antigen specific proliferation (MTT) and respiratory burst activity (NBT) were determined on pronephros cells at 7, 14, 21 and 28 days post-injection. Provisional results indicate that antigen specific proliferation was significantly higher than control in vaccine group at 7 days. The respiratory burst activity in all injected groups was higher than the control after 14 days.

Additionally, serum analysed for antibody titre against *Aeromonas hydrophila* and the alternative complement pathway (ACH50) activity at 7, 14, 21 and 28 days post-injection. A significant increase of antibody titre level observed in all injected groups compare to control at 14 days and the combination of vaccine and adjuvant group boost the antibody titre level one week earlier compare to the vaccine group at 28 days. The ACH50 in all injected groups was significantly higher than the control after 7 days. This preliminary study gives the possibility of using this formulated carbohydrate as an adjuvant in carp.

T.E.M. AND BIOMOLECULAR DETECTION OF RICKETTSIALES IN TISSUES OF RAINBOW TROUT AND THEIR POTENTIAL ROLE AS RED MARK SYNDROME (RMS) ETIOLOGICAL AGENTS

M. GALEOTTI[1]*, M. MANZANO[1], F. CECCHINI[1], D. VOLPATTI[1], C. BULFON[1], P. BERALDO[1], G. ROSSI[2], G. MAGI[2]

[1]*Department of Food Science, Veterinary Pathology Section, University of Udine, Udine, Italy*
[2]*School of Bioscience and Veterinary Medicine, University of Camerino, Camerino, Italy*

RMS and US SD are skin disorders of farmed rainbow trout occurring in many European countries and US. The diseases aetiology is still uncertain. In spite of specific investigations identifying the presence of RLOs related DNA in tissues of US SD and RMS affected individuals (Lloyd *et al*., 2008; Metselaar *et al.,* 2010), these pathogens have never been morphologically detected in samples.
The present study represents the prosecution of previous researches carried out in the past years on Italian RMS outbreaks, finalized to comprehend the disease pathogenesis and causative agent. In detail we performed histological, T.E.M. and bimolecular analysis on numerous skin and spleen samples collected from RMS symptomatic fish, classified accordingly to the diagnostic criteria published by Oidtman *et al*. (2013).
The histological evaluation underlined, in all skin lesions, the presence of the typical lymphocyte /macrophage infiltration involving various layers of this organ, still there was no evidence of microbial agents. T.E.M. observation revealed the presence of intra-cytoplasmic electron dense bacteria frequently surrounded by a clear halo, within macrophages, fibroblasts and erythrocytes. The microorganisms were oval or rod shaped, displaying a size ranging from 400 to 700 nm in length and 100 to 200 nm in width. They showed a thin cell wall lining an electron dense granular cytoplasm, that in some cases showed a thread like structure of DNA filled matrix located at the one pole of the bacterial body.
Biomolecular insights allowed to detect a DNA related to Rickettsiales. The concomitant T.E.M. and PCR findings in RMS affected rainbow trout allow us to strongly suspect a bacteria belonging to Rickettsiales order as the causative agent of this disease. Further investigations are still necessary to clear the role of these bacteria in the pathogenesis of rainbow trout RMS.

PATHOLOGICAL EFFECT OF *NOCARDIA* SPP. INFECTION IN MEAGRE (*ARGIROSOMUS REGIUS*)

B. VEGA[1]*, F. ACOSTA[1], C. RIBEIRO[2], M.A. RUIZ[2], D. MONTERO[2], M.J. CABALLERO[2]

[1]*Grupo Inmunología Veterinaria y Producción Animal, ULPGC, Arucas, Spain*
[2]*Grupo de Investigación en Acuicultura, ULPGC, Spain*

Nocardiosis caused by *Nocardia* sp. in fish was first described by Rucker (1949) as *Streptomyces salmonicida* infecting sockeye salmon (*Oncorhynchus nerka*). Nocardiosis is a systemic bacterial disease caused by a Gram-positive, partially acid-fast, aerobic, filamentous bacterium. Typical disease signs include granulomas in gills, spleen, kidney and liver with or without multiple skin ulcers/nodules.. In our work we used a strain of Nocardia spp. isolated from meagres (*Argirosomus regius*). We conducted a challenge using 200 fish weighing approximately of 95gr, and infected with our Nocardia at doses from 10^9-10^2 cfu/ml, 1ml injected intraperitoneally. The deaths were recorded and organ samples were taken for microbiological and histopathological analysis at five sampling points. Of the total injected fish, 15 fish died in the higher doses (10^9 and 10^8 cfu/ml). Histologically, kidney granulomas were observed a week after the inoculation in 10^9dose, while granulomas in fish inoculated with lower doses were observed for the first time three weeks later. The microbiological results show that both of dead fish and slaughtered have isolated and identified *Nocardia* spp. from liver, spleen and kidney. These results show the pathological evolution of an experimental Nocardia infection in meagre.

IRON UTILIZATION AND SIDEROPHORE PRODUCTION BY *RENIBACTERIUM SALMONINARUM* ISOLATED FROM DISEASED ATLANTIC SALMON (SALMO SALAR) IN CHILE

J. BETHKE*, M. POBLETE-MORALES, R. IRGANG, R. AVENDAÑO-HERRERA

Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Viña del Mar, Chile
Interdisciplinary Center for Aquaculture Research (INCAR), Concepción, Chile

Renibacterium salmoninarum is the causative agent of Bacterial Kidney Disease (BKD) and a Gram-positive bacterium that has been recognized as a major salmonid pathogen for over 70 years, because it is one of the few vertebrate bacterial pathogens known to be vertically transmitted making the efficient combat of the disease difficult. Although the pathogenicity of *R. salmoninarum* has been associated with hydrophobicity and a secreted protein of 57 kDa, the virulence mechanisms are still poorly understood. Iron is an essential element in a variety of metabolic cellular pathways and an important mechanism of virulence. Until now, little is known about the ability to take up iron from the host during BKD infection, and only an iron reductase has been described. This work represents the first evidence of the presence of iron uptake mechanisms in this bacterial fish pathogen. Thirty-two Chilean isolates obtained from Atlantic salmon (*Salmo salar*) and the type strain DSM 20767^T were examined. All of them were able to grow in the presence of the chelating agent 2,2'-dipiridyl up to 300 μM. Production of siderophores in all *R. salmoninarum* was corroborated by chrome azurol S assays, and *in silico* analysis of the DSM 20767^T genome (RefSec: NC_010168) revealed the presence of an enterobactin siderophore pathway involved in iron uptake regulation and other pathways like hemin and a variety of ABC like transporters that could be involved. Based on biochemical and genetic homogeneity of *R. salmoninarum* and the results of the siderophore production assays, two representatives Atlantic salmon isolates and the type strain were growth in iron-deficient media with different iron sources such as hemin, haemoglobin, ferric ammonic citrate, ferric chloride and ferrous sulfate. In this study, we sought to get a first insight into the mechanisms that *R. salmoninarum* possess for iron assimilation from the host tissues. The characterization of these iron acquisition pathways may be crucial for the development of alternative and more efficacious therapeutics and vaccines, due to current therapies and preventative strategies are only marginally effective in preventing this disease.

Financed by FONDAP 15110027, FONDECYT 1150695 and PhD scholarship from the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT, Chile).

IN VITRO STUDY OF ADHERENCE AND INVASION OF *PHOTOBACTERIUM DAMSELAE* SUBSPECIES *PISCICIDA* IN SAF-1 CELL LINE BY CONFOCAL AND ELECTRON MICROSCOPY

F. ACOSTA[1]*, B. VEGA[1], J. BRAVO[1], L. ROMÁN[1], O. ESCUELA[1], J. VEGA[2], F. MADRAZO[3], J. RAMOS-VIVAS[4]

[1]*Grupo de Inmunología Veterinaria y Producción Animal, Universidad de Las Palmas de Gran Canaria, Arucas, Spain*
[2]*Instituto Universitario de Sanidad Animal, Universidad de Las Palmas de Gran Canaria, Arucas, Spain*
[3]*Laser Microscopy Unit, Fundación Instituto de Investigación Marqués de Valdecilla (IDIVAL), Santander, Cantabria, Spain*
[4]*Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla, Instituto IDIVAL, Santander, Cantabria, Spain*

Photobacterium damselae subspecies *piscicida* (PHDP), previously known as *Pasteurella piscicida* is an important pathogen affecting fish species in Europe, Japan, USA and the Mediterranean coast. Information on the pathogenesis of pasterelosis is scarce. Furthermore, interaction with no phagocytic cells of PHDP in seabream not been studied previously. For this work we already have a set of well-characterized strains PHDP C2 strain, isolated in our laboratory from outbreaks of pasteurellosis in the open sea. Stable fibroblast cell line SAF-1 has been previously characterized by Spanish researchers, had been used for infections. Studies of adhesion and internalisation were performence by confocal and electron microscopy. After studying fluorescence we observed phenomena of adhesion and internalization after 5 min of contact of bacteria to cells with precencia of attached bacteria and an event internal bacteria. On examination of the infected cells are cells that exhibit modification phenomena its cytoplasmic the contact or proximity of the bacteria membrane, as reflected in the form of extension that will encompass bacteria and introduce them inside the cell, internalization of the bacteria that is within a vacuolar structure is also evident. In our observations of bacteria we observed the presence of polar pili by SEM, TEM.

THE USE OF "TAXO-GENOMICS" TO CLARIFY THE NOMENCLATURE AND TAXONOMICAL STATUS OF FISH PATHOGENIC *FRANCISELLA* SPP.

J.G. RAMÍREZ-PAREDES[1]*, E. SOTO[2], C. ÖHRMAN[3], K. JOLLEY[4], M. MAIDEN[4], H.J. BUSSE[5], R.H. RICHARDS[1], D.J. COLQUHOUN[6], P. LARSSON[7], A. ADAMS[1]

[1]*Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling, Scotland, UK*
[2]*School of Veterinary Medicine, University of California-Davis, California, USA*
[3]*Swedish Defence Research Agency (FOI), CBRN Defence and Security, Umeå, Sweden*
[4]*Department of Zoology, University of Oxford, Oxford, England, UK*
[5]*Institute for Microbiology and Hygiene, University of Veterinary Medicine, Vienna, Austria*
[6]*Section for Bacteriology, Norwegian Veterinary Institute, Oslo, Norway*
[7]*Laboratory Medicine, Clinical Genetics/Pathology, Umeå University Hospital, Umeå, Sweden*

Francisella noatunensis (*Fn*) is a facultative intracellular bacterium causative agent of "piscine francisellosis", a disease that affects several marine and fresh water fish species worldwide including salmon, cod and several cichlids. In this study the genome of 11 *Fn* strains was sequenced using high-throughput sequencing technology (Illumina-HiSeq® platform), annotated and used in comparative analysis with other publicly available *Francisella* spp. strains. The genomic analyses included: 16S rRNA gene similarity and phylogeny, whole genome average nucleotide identity (wg-ANI_m), genome to genome distance (GGD), wet lab and *in silico* DNA-DNA hybridization (DDH), multilocus sequence analysis (MLSA), whole genome G+C content and phylogeny, and whole genome ribosomal multi locus sequence typing (rMLST). Additionally the phenotypic and chemotaxomic characteristics of 32 *Fn* strains and the type strain of the closely related *F. philomiragia* were investigated using metabolic fingerprinting (Biolog® GN2 plates) and chemotaxonomic analyses (fatty acids methyl ester profiling, quinone system profiling, polyamine analyses and polar lipids profiling). In total 47 strains were phenotypically and/or genetically characterised. The results of this study indicated a misplacement within the *taxon Fn* and suggested the creation of the new species: *F. orientalis* (by elevating the rank of *F. noatunensis* subsp. *orientalis* to the species level) to allocate strains from diseased fish farmed in tropical aquaculture or captured in warm water environments and separate them from the *taxon F. noatunensis*. What is more, the present findings clearly supported the creation of a new subspecies within *Fn*, for which the name *Francisella noatunensis* subsp. *chilense* was given, to separate the isolates recovered from moribund salmon farmed in Chile from those isolated from wild and farmed cod and farmed salmon in Northern Europe. In addition, an amended description of *F. noatunensis* subsp. *noatunensis* (a subspecies automatically created with the description of *F. noatunensis chilense*) will be presented.
It is proposed that the criterions here presented for the description of new species and subspecies of fish pathogenic *Francisella* spp. should be adopted for further research in the taxonomy of the genus *Francisella*.

SUSCEPTIBILITY OF COMMON CARP AND SUNFISH TO *FRANCISELLA NOATUNENS* SUBSP. *ORIENTALIS*

E. LEWISCH*, S. MENANTEAU-LEDOUBLE, M. EL-MATBOULI

Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria

Francisella noatunensis subsp. *orientalis* (Fno) is a well known pathogen of farmed and wild fish and shellfish species. These bacteria have recently received increased attention as a pathogen of ornamental tropical fish. To investigate possible risks of transmission from warm water ornamental fish to native fish species, infection trials were conducted under laboratory controlled conditions. The bacterial strain originated from a natural outbreak of francisellosis in a breeding stock of Malawi cichlids. Fish species for the infection trials were chosen based either on economic importance (common carp, *Cyprinus carpio*) or genetic relation to the cichlids (sunfish, *Lepomis gibbosus*). Fish were challenged by intraperitonal injection. Signs of disease and mortalities could only be observed among infected sunfish within the first three days post infection. Genome equivalents of Fno were detected by PCR in organ homogenates of 28/41 challenged sunfish until 143 days p.i. and 5/41 challenged carp until 139 days p.i. and in gills of selected sunfish. Granulomas were observed in spleens of two cohabitated sunfish and kidney of one challenged carp, but all three fish tested negative for *Francisella*-like bacteria (FLB) specific PCR. Fno could be recovered from kidney and spleen of one carp. Identity of the bacteria was demonstrated by Gram stain and FLB specific PCR. Spleen and kidney tissue of this carp tested positive in FLB specific PCR. While FLB specific PCR was positive from gill tissues of selected fish, indicating bacteriaemia, transmission to cohabitated fish could not be demonstrated. Under the specific setting of the experiment, sunfish seemed to be sensitive to the applied bacteria strain, but fish, which did not die within the first three days p.i. overcame the infection during the course of the experiment. In carp, the infection did not impact health and survival.

ACTIVATION OF RAINBOW TROUT SKIN CD8$^+$ DENDRITIC-LIKE CELLS IN RESPONSE TO ANTIGENIC EXPOSURE

A.G. GRANJA[1], E. LEAL[1], R. CASTRO[1], B. ABÓS[1], U. FISCHER[2], C. TAFALLA[1]*

[1]*Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos 28130, Madrid, Spain*
[2]*Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany*

Jawed fish are the first evolutionary group in which adaptive immune responses are present, and the elements implicated in antigen processing and presentation exist in most members of this animal group. Although there is evidence for the existence for dendritic cells (DC) in teleost fish, the lack of adequate immunological tools has hampered a full phenotypic and functional characterization of specific DC subsets. We have very recently identified in rainbow trout (*Oncorhynchus mykiss*) a subpopulation of leukocytes co-expressing MHC II and CD8a, which resembles mammalian CD8$^+$ dendritic cells. These cells are mainly present in mucosal tissues, and most abundant in the skin, where they represent 1.2% of total leukocytes. In the current study, we have analyzed the *in vivo* response of this trout skin CD8$^+$ DC-like subpopulation. After water bath exposure with zymosan particles, the number of CD8$^+$ DCs was significantly augmented in the skin, along with an increase of membrane MHC II. Additionally, skin CD8$^+$ DCs up-regulated the expression of BAFF, IFN-g and CD83 in response to zymosan. Interestingly, a similar response was seen in the spleen (increase of the number of CD8$^+$ DCs and increase of membrane MHC II), which might indicate that the local response triggered in the skin might be translated by CD8$^+$ DCs to central lymphoid organs. When fish were bath-exposed to viral hemorrhagic septicemia virus (VHSV), the number of CD8$^+$ DCs was increased after infection in a similar trend to that seen with zymosan particles. In parallel, skin CD8$^+$ DCs up-regulated the expression of BAFF and CD83, but also the expression of MHC I and CD40. In addition, the up-regulation of the expression of IFN-g after VHSV-treatment was considerably higher than that seen with zymosan. These results shed light on how a specific subset of skin-resident DC-like population triggers antiviral responses in fish mucosal tissues.

RAPID PROLIFERATION RATHER THAN SURVIVAL IN BLOOD MAY BE A REQUIREMENT FOR STREPTOCOCCAL PATHOGENESIS IN BARRAMUNDI (*LATES CALCARIFER*): UNDERLYING MECHANISMS OF IMMUNE EVASION

K.A. MASTERMAN*, A.C. BARNES

The University of Queensland, Brisbane, Australia

Streptococcal infections caused *by S. agalactiae* and *S. iniae* result in widespread mortalities in farmed fish in warm-temperate and tropical waters throughout the world. Following infection, Streptococcus rapidly migrate to the bloodstream where they multiply and disseminate before colonising the CNS resulting in meningitis and death. The first responder immune cells in blood are neutrophils, therefore pathogens that are able to multiply in the blood must be capable of evading or subverting host neutrophils. Barramundi (*Lates calcarifer*) are an economically and culturally important euryhaline fish farmed throughout tropical and sub-tropical Asia and Australia. *S. iniae* is a major pathogen of barramundi. Contrastingly, there are no reports of *S. agalactiae* infections in the same species, even though epizootic disease attributed to *S. agalactiae* occurs in other co-located farmed and wild fish species. To better understand how *S. iniae* circumvents the neutrophil response in barramundi, we compared survival and growth of *S. iniae* with a strain of *S. agalactiae,* highly virulent in grouper*,* in barramundi blood. We show that *S. agalactiae* is unable to multiply, whereas *S. iniae* rapidly proliferates, doubling in less than 30 min. The polysaccharide capsule is major virulence factor and is the most common target for vaccine development. Use of a capsular defective strain of *S. iniae* in the blood-bactericidal assay did not abrogate microbial survival, however the rapid proliferation was reduced suggesting that while the capsule is important for bacterial colonisation it is not the only means used to evade neutrophils. The precise mechanisms by which *S. iniae* is able to evade neutrophils and proliferate so rapidly in barramundi blood is not yet known. Ongoing studies seek to determine how *S. iniae* evades, inhibits or kills these phagocytic sentinels, and whether specific capsular gene knockout can improve host cell bacterial recognition and clearance.

PARASITISM AFFECTS VACCINE EFFICACY AGAINST *STREPTOCOCCUS INIAE* IN NILE TILAPIA

C.A. SHOEMAKER[1]*, M.L. MARTINS[2], D. XU[1]

[1]*United States Department of Agriculture-Agricultural Research Service, Aquatic Animal Health Research Unit, Auburn, AL, USA*
[2]*Aquatic Organisms Health Laboratory, Federal University of Santa Catarina, Florianópolis, Brazil*

Tilapia culture worldwide is estimated to be US$ 5 billion and is important to domestic and global food security. Parasites and bacteria co-occur in both extensive and intensive production of tilapia. The effect of parasitism on vaccine performance in fish is little studied. The objective of this study was to determine if parasitism of tilapia affected vaccine efficacy. Antibody level and survival of Nile tilapia vaccinated with a modified *Streptococcus iniae* bacterin were compared among non-parasitized fish, fish parasitized by *Trichodina heterodentata* and *Gyrodactylus cichlidarum*, and fish parasitized by *T. heterodentata, G. cichlidarum* and *Ichthyophthirius multifiliis* (Ich). Among vaccinated fish, fish free from parasites (*Trichodina, Gyrodactylus* and Ich) had the highest antibody level (0.43, SE=0.14). Significantly ($p < 0.05$) lower anti-*S. iniae* antibody was noted in parasitized vaccinated fish (0.30, SE=0.08). Among the vaccinated treatments post challenge, fish parasitized by *Trichodina, Gyrodactylus* and Ich showed the lowest survival (80.0%, SE=10.0), significantly ($p<0.05$) lower than vaccinated fish free from parasites (97.5%, SE=2.5) or parasitized by *Trichodina* and *Gyrodactylus* (95.0%, SE=5.0). Following challenge with *S. iniae*, non-vaccinated fish free from parasites showed higher survival (47.5%, SE=2.5) than non-vaccinated fish parasitized by *Trichodina* and *Gyrodactylus* (37.5%, SE=2.5). Non-vaccinated fish parasitized by all 3 parasites showed the lowest survival (27.5%, SE=2.5) post challenge. Relative percent survival (RPS) demonstrated a decrease in vaccine performance for the group of fish that were parasitized with *Trichodina* and *Gyrodactylus* and Ich. RPS was 72% compared to 95 and 92%, respectively, in the other vaccinated treatments following challenge. This study demonstrated a reduction in vaccine performance in parasitized tilapia and highlights the importance of monitoring or controlling parasite levels in the aquaculture setting. Limiting parasitic infection should be considered in fish health management as a strategy to enhance vaccine effectiveness.

CHOLESTEROL/FARNESYL DIPHOSPHATE SYNTHASE AS SUITABLE TARGETS FOR HOST ANTIVIRAL AND TREATMENT STRATEGIES.

M. ADAMEK[1]*, G. BROGDEN[1, 2], F.E. MÜLLER[1], H.Y. NAIM[2], D. STEINHAGEN[1]

[1]*Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2]*Department of Physiological Chemistry, University of Veterinary Medicine, Hannover, Germany*

Cholesterol is essential for building and maintaining cell membranes; being a main component of lipid rafts and modulating membrane fluidity. Cholesterol/lipid rafts are critical for several steps in the viral replication cycle, especially for enveloped viruses. Cholesterol biosynthesis in the cell is a product of the mevalonate pathway. This complex reaction cascade requires the activity of enzymes such as HMG-CoA synthase and reductase, mevalonate kinase and farnesyl diphosphate synthase (FDPS).
Our RT-qPCR analysis showed that this pathway can be modulated under viral infection. Both *Cyprinid herpesvirus 3* (CyHV-3) and *Spring viraemia of carp virus* (SVCV) significantly down-regulated the mRNA expression of FDPS in common carp fibroblasts. This down-regulation coincided with an up-regulation of type I interferon during SVCV but not in CyHV-3 infection, which is not inducing an IFN response.
In order to confirm the importance of cholesterol-rich lipid rafts in the replication cycle of enveloped piscine viruses we used methyl-beta-cyclodextrin (MβCD), which is capable of removing up to 70% of the cholesterol from cell membranes and thus disrupting lipid rafts. With this treatment we showed in several assays, including virus titration, immunocytochemistry and (RT)-qPCR analysis, that cholesterol is crucial for CyHV-3 entry into and egress from the host cell. We also confirmed that MβCD treatment had a similar effect on SVCV infectivity.
To evaluate the significance of FDPS for virus replication, cells were treated with zoledronic acid, which is a very strong FDPS inhibitor. Surprisingly, this treatment had an extremely strong dose dependent effect on CyHV-3 replication and lowered the virus titer by up to 1000x. Its effect on SVCV replication was noticeably lower (titer reduction <10x). However, the treatment of cells with zoledronic acid did not have such a drastic influence on cholesterol levels in cell membranes as MβCD, therefore the antiviral effect could be exerted via blocking protein prenylation.
These results might have fundamental connotations for further research on innate immune responses, such as type I interferons, which are influencing the formation of lipid rafts in host cells. Furthermore the cholesterol synthesis pathway could be a suitable target for treatment or prevention of viral infections in fish.

RENIBACTERIUM SALMONINARUM AND INNATE IMMUNITY IN ATLANTIC SALMON (*SALMO SALAR* L.) AND ARCTIC CHARR (*SALVELINUS ALPINUS* L.)

S. GUDMUNDSDOTTIR*, B.TH. BRAGASON, I.Ö. ARNASON

Institute for Experimental Pathology, University of Iceland, Keldur, 112-Reykjavik, Iceland

The aim was to compare innate immune reactions of Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.) during infection with *Renibacterium salmoninarum.*
Salmon fingerlings were reared in full salinity and Arctic charr fingerlings in 15‰ salinity. Fingerlings of both species were infected by injecting $5x10^6$ bacteria intra-peritoneally (i.p.). Controls, reared in the same tanks, received PBS. Both species were sampled before injection and after 1, 4, 7, 10, 14 and 22 days.
The course of infection was monitored by testing head kidney samples in ELISA that detects bacterial antigens and in snPCR test that detects bacterial DNA. To study the innate immune reactions, primers for MHC-I, cathelicidin, NADPH and TGF-β in addition to ELF-1 (reference gene) were designed so that they detected homologous target sequences in the genes for both species. RNA was isolated from head kidney and analysed by RT-qPCR (SYBR green) to determine the gene expression.
ELISA values in injected fish were positive as of day one after injection and mean ELISA values were significantly higher for Arctic charr on days 7, 14 and 22. One control salmon was positive in ELISA on day 22. On day one, one injected salmon was positive in snPCR and on day four, 5 of 8 salmon and 3 of 8 Arctic charr samples were positive in snPCR.
All target genes were expressed in head kidney of both species, sampled before injection. The i.p. infection significantly upregulated the gene expression of cathelicidin, NADPH and TGF-β in both species, when compared to controls, but MHC-I was only upregulated in salmon. A significant effect on TGF-β gene expression was observed in samples from salmon controls (co-habitation) at the end of the experiment.
Both species react with upregulation of cathelicidin that is bactericidal and NADPH that plays a part in respiratory burst. Upregulation of TGF-β probably reflects enhancement of anti-inflammatory events. Why MHC-I, necessary for presentation of intracellular pathogens, is upregulated in salmon but not in Arctic char is unexplained. A longer time for observation might be needed. Using the same primers for both species was done in order to make the comparison more precise.

RATIONAL DESIGN OF A SAFE AND EFFICACIOUS ATTENUATED RECOMBINANT VACCINE AGAINST CYPRINID HERPESVIRUS 3 USING PROKARYOTIC MUTAGENESIS AND IN VIVO IMAGING SYSTEM

M. BOUTIER[1], M. RONSMANS[1], P. OUYANG[1], G. FOURNIER[1], K. RAKUS[1], A. RESCHNER[1], A. DAVISON[2], F. FARNIR[1], C. BAYROU[1], F. LIEFFRIG[3], D. DESMECHT[1], A. VANDERPLASSCHEN[1]*

[1]*University of Liège, Liège, Belgium*
[2]*University of Glasgow, Glasgow, United Kingdom*
[3]*CER Groupe, Marloie, Belgium*

Cyprinid herpesvirus 3 (CyHV-3) is causing severe economic losses worldwide in common and koi carp industries, and a safe and efficacious attenuated vaccine compatible with mass vaccination is needed.
We produced single deleted recombinants using prokaryotic mutagenesis. When producing a recombinant lacking open reading frame 134 (ORF134), we unexpectedly obtained a clone with additional deletion of ORF56 and ORF57. This triple deleted recombinant replicated efficiently in vitro and expressed an in vivo safety/efficacy profile compatible with use as an attenuated vaccine. To determine the role of the double ORF56-57 deletion in the phenotype and to improve further the quality of the vaccine candidate, a series of deleted recombinants was produced and tested in vivo. These experiments led to the selection of a double deleted recombinant lacking ORF56 and ORF57 as a vaccine candidate. The safety and efficacy of this strain were studied using an in vivo bioluminescent imaging system (IVIS), qPCR, and histopathological examination, which demonstrated that it enters fish via skin infection similar to the wild-type strain. However, compared to the parental wild-type strain, the vaccine candidate replicated at lower levels and spread less efficiently to secondary sites of infection. Transmission experiments allowing water contamination with or without additional physical contact between fish demonstrated that the vaccine candidate has a reduced ability to spread from vaccinated fish to naïve sentinel cohabitants. Finally, IVIS analyses demonstrated that the vaccine candidate induces a protective mucosal immune response at the portal of entry.
Thus, the present study is the first to report the rational development of a recombinant attenuated vaccine against CyHV-3 for mass vaccination of carp. We also demonstrated the relevance of the CyHV-3 carp model for studying alloherpesvirus transmission and mucosal immunity in teleost skin.

Part of this work has been recently published in PLoS Pathog. 2015 Feb 20;11(2):e1004690

ABILITY OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS TO EVADE THE PROTECTIVE IMMUNE RESPONSE INDUCED IN RAINBOW TROUT BY DNA VACCINATION

D. SEPÚLVEDA*, N. LORENZEN

Aarhus University, Aarhus, Denmark

Viral haemorrhagic septicaemia virus (VHSV) is a negative strand RNA virus, which belongs to the genus *Novirhabdovirus* within the family *Rhabdoviridae*. This virus is the causative agent of VHS, a serious disease in rainbow trout and other economically important fish species. The DNA vaccine encoding the viral glycoprotein has been successful as an experimental prophylactic treatment against this disease, inducing a strong innate and adaptive immune response. However, since RNA viruses are known to possess high variability and adaptation capacity, this work aims to evaluate whether VHSV is able to evade the protective immune response induced by the DNA vaccination.

The evasion capacity of VHSV was evaluated through two approaches. First, *in vitro* approach focus on isolate VHSV variants in cell culture able to escape the neutralizing antibodies of serum from fish immunized with the DNA vaccine. And second, the *in vivo* approach to evaluate the possibility to isolate a VHSV variant able to evade the protection of the fish vaccinated with the DNA vaccine. The experiments comprise repeated serial passages of the highly pathogenic VHSV isolate DK3592b (parental virus) in EPC cells in presence of neutralizing fish serum for the *in vitro* approach, and in rainbow trout injected with the DNA vaccine for the *in vivo* approach.

For the *in vitro* approach, the virus isolated after 11 passages in EPC cell was as sensitive as the parental virus to the treatment with neutralizing antibodies from serum. For the *in vivo* approach, after successive passages of infection, the comparison between the passaged viruses and the parental virus showed that all of them caused low mortality rates in vaccinated fish. Further analysis of the survivor vaccinated fish revealed that all viruses were able to persist in only few vaccinated fish. However, this was enough to spread the infection to cohabitant naïve fish.

The DNA vaccine triggers a broad range of protective mechanisms including both the innate immune response and the humoral and cellular arms of the adaptive immune response. This might explain why it was not possible for the virus to evade the vaccine-induced protection against disease. Since the vaccine does not protect against infection and asymptomatic vaccinated fish can be infected and spread the virus to naïve cohabitants, it is important not to transfer vaccinated fish from geographical regions with VHSV into VHSV free zones.

FRESHWATER TREATMENTS IN THE UK RAINBOW TROUT INDUSTRY: IS THERE LIFE AFTER FORMALIN?

N. TAYLOR, D.W. VERNER – JEFFREYS*

Cefas Weymouth Laboratory, Weymouth, UK

Availability of effective treatments for control of infectious diseases is a critical requirement of the UK rainbow trout industry. Producers, vets and health professionals were surveyed to determine what the main diseases are affecting them and what treatments they using. They confirmed that production was constrained by a limited group of common diseases that affected rainbow trout producers in England and Scotland. These included rainbow trout fry syndrome (RTFS) caused by the bacterium *Flavobacterium psychrophilum*, white spot disease caused by the endoparasite *Ichthyophonus multifiliis*, enteric redmouth disease (ERM) caused by the bacterium *Yersinia ruckeri*, proliferative kidney disease caused by *Tetracapsuloides bryosalmona*, red mark syndrome (RMS) and bacterial gill disease (BGD).

The main treatments available to control these conditions were limited, with florfenicol reportedly used by all producers to control RTFS, formalin used extensively to control white spot and a range of parasites and chloramine T to treat bacterial gill disease. ERM was mainly controlled by vaccination, particularly via dip vaccination of fry with the Relera dual antigen vaccine. Other licensed antibiotics (oxytetracycline, amoxicilin and oxolinic acid) were used to treat sporadic outbreaks of ERM, in fish where vaccine protection had waned, and furunculosis.

The major reliance of the industry on florfenicol and formalin was concerning. Firstly there were limited identified alternatives to control RTFS in the event of RTFS-causing strains of *F. psychrophilum* developing resistance to florfenicol. There is also pressure at an EC level to withdraw formalin from sale as a biocide. Possible alternatives to the use of formalin products purchased for biocidal applications were reviewed in the event of their withdrawal from sale. For control of white spot it may be possible to use a licensed product marketed in Spain for the control of parasites of turbot under the veterinary cascade. The bronopol containing medicine Pyceze^{TM} is one identified alternative that may be used. Where systems can be engineered to allow its use, Salt (sodium chloride), either via low concentration continuous dosing for several days, or short duration high concentration flushes is also a potential treatment. Practical issues with regards either maintaining low concentrations of salt, or dealing with high concentration effluents, may limit the use of this treatment strategy though. Danish producers are also reportedly trialing the use of peracetic acid. For control of some ectoparasites, particularly flukes (e.g. trichodina), praziquantel, either as a water-based or in feed treatment, may also be an option to explore.

Selection of any alternative treatments should be guided by whether they are likely to be readily useable. In this regards, products that already have approval for use in food animal production, either as biocides, feed additives or as medicines should be preferred in the first instance.

ACUTE TOXICITY OF PERACETIC ACID TO VARIOUS FISH SPECIES

D.L. STRAUS[1]*, C.K. LEDBETTER[1], B.D. FARMER[1], T. MEINELT[2], L.-F. PEDERSEN[3]

[1]*U.S. Department of Agriculture, Agricultural Research Service, Harry K. Dupree - Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, USA*
[2]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*
[3]*Technical University of Denmark, North Sea Research Centre, Hirtshals, Denmark*

Peracetic acid (PAA; also called peroxyacetic acid) is a promising disinfectant in the US aquaculture industry to control parasites and fungus. It is a stabilized mixture of acetic acid and hydrogen peroxide that does not leave dangerous residues in the environment when it breaks down as most compounds do. The US Environmental Protection Agency (EPA) first registered PAA as an antimicrobial in 1985 for indoor use on hard surfaces (hospitals). EPA registrations now include: sanitation in food/beverage plants, agricultural premises, wineries/breweries, greenhouse equipment, and animal housing. It is also used to prevent bio-film formation in paper/pulp industries and as a disinfectant for wastewater treatment. PAA is used extensively in Europe, and our international collaborations have studied its effectiveness to many pathogens. However, there is a lack of data on its toxicity to fish.

This study determined the acute toxicity of PAA to 12 fish species in well water. The experiments were designed to provide the 24 h LC50, LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values for each species at ~ 23°C. Ten fish were placed in static aquaria containing 10 L of well water. Each experiment consisted of 6 PAA concentrations and an untreated control (n=3). Water chemistry for the well water was: pH = 7.5, total alkalinity = 200 mg/L, total hardness = 125 mg/L. The mean LC50 value for all species tested was 5.3 mg/L PAA with the range of 2.8 mg/L to 9.3 mg/L. Black fathead minnows and blue tilapia were most and least sensitive, respectively. The mean LOEC value for all species tested was 3.7 mg/L PAA with the range of 1.9 mg/L to 5.8 mg/L. The immediate impact of this research is to understand the toxicity variance among species and ultimately to determine safe and effective therapeutic treatments.

GROWTH INHIBITION OF *AEROMONAS SALMONICIDA* AND *YERSINIA RUCKERI* BY DISINFECTANTS CONTAINING PERACETIC ACID

T. MEINELT[1,]*, S. BEHRENS[1], L.-F. PEDERSEN[3], D. LIU[1], D.L. STRAUS[2]

[1]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*
[2]*USDA Aquaculture Research Center, Stuttgart, USA*
[3]*DTU Aqua, Section for Aquaculture, Hirtshals, Denmark*

Peracetic acid (PAA) is an agent used for disinfection in aquaculture. PAA contributes to sustainable aquaculture, because it releases no harmful residue in the environment. However, there is lack of guideline about the effective application of different PAA products against various pathogens in practical aquaculture disinfection. The aim of our study was to compare the effectiveness of six commercial PAA products with different molecular PAA: H_2O_2 ratios to reduce bacterial growth of *A. salmonicida* and *Y. ruckeri*, and to determine effective concentrations and exposure times.
All products reduced colony forming units (CFUs) of *A. salmonicida* and *Y. ruckeri*. Products with a higher concentration of PAA (versus H_2O_2) did inhibit growth better than products with lower PAA and higher H_2O_2 concentrations; H_2O_2 is not the driving force in the reduction of *A. salmonicida* and *Y. ruckeri* growth by PAA *in vitro*. The practical application of the products with high PAA concentration should be prioritized if these pathogens are diagnosed.

CONTROL OF SAPROLEGNIASIS ON SUNSHINE BASS EGGS WITH COPPER SULFATE

D.L. STRAUS[1]*, B.D. FARMER[1], C.K. LEDBETTER[1], B.H. BECK[1], R.S. WILLIAMS[2], M.L. CLARK[2], T.M. FREEZE[2]

[1]*U.S. Department of Agriculture, Agricultural Research Service, Harry K. Dupree - Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, USA*
[2]*Keo Fish Farms, Inc., Keo, Arkansas, USA*

A major obstacle to successful hatchery production is water-mould (Saprolegniasis) growth on eggs. Copper sulfate ($CuSO_4$) is commonly used to control *Saprolegnia* species in channel catfish hatcheries that use troughs, but the effectiveness of it on fish eggs hatched using different systems was not known. Therefore, the range-finding study consisted of three $CuSO_4$ concentrations (10, 20, and 40 mg/L) and an untreated control (n=3).
Female white bass *Morone chrysops* and male striped bass *M. saxatilis* were spawned, and eggs were transferred immediately to 6 L McDonald jars. After treatments to remove adhesiveness and to disinfect the eggs as in a typical hatchery, the 1 mm eggs were counted with an optoelectronic XperCount™ enumerator and transferred to each hatching chamber of our experimental system. Water flow maintained the rolling action of the eggs per industry standards. Because eggs start hatching after 2 d, treatment began immediately the afternoon of spawning with a 10 min aerated, static bath and was repeated morning and afternoon on Day 2. Eggs were not treated after hatching began. The 3 – 4 mm fry were counted with the XperCount™ on Day 4.
Water-mould samples from the controls were identified as *Saprolegnia ferax*. Saprolegniasis was severe in the untreated controls (28% survival). Very little water-mould was present in treatments receiving 10 mg/L $CuSO_4$ (32% survival) or higher. The best survival was at 40 mg/L $CuSO_4$ (50% survival); however, the 20 mg/L $CuSO_4$ treatment (46% survival) gave similar results and allows for a greater margin of safety. We developed an *in vitro* assay that confirmed maximum Saprolegniasis inhibition was achieved at 20 mg/L $CuSO_4$.

EFFECT OF IMMUNIZATION TEMPERATURE AND TIME ON THE ABILITY OF ATLANTIC SALMON TO ELICIT SPECIFIC ANTIBODY RESPONSES AND PROTECTION AGAINST *MORITELLA VISCOSA*

R. THORARINSSON[1]*, D. VOLLSTAD[1], A. RAMSTAD[2], P. MIDTLYNG[3, 4]

[1]*Elanco Animal Health, Bergen/Tromsø, Norway*
[2]*VESO Vikan, Namsos, Norway*
[3]*School of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway*
[4]*Aquamedic AS, Lörenskog, Norway*

PIT tagged Atlantic salmon (*Salmo salar*) pre-smolts of uniform origin were split into equal tank populations, acclimated to 3 different water temperatures (15, 8 or 4ºC, respectively), fed *ad libitum*, and immunized. Vaccine groups were i.p. administered with commercially available, oil adjuvanted 6-component vaccines containing *Moritella viscosa* antigen, while a control group was sham-vaccinated with phosphate buffered saline (PBS). Two parallel tanks with all immunisation groups were formed per temperature regime. Vaccinations were performed 43, 73 or 130 days before the predetermined challenge date, so that each temperature regime would accumulate a uniform 600 degree-day immunisation period. After smoltification induced by photoperiod manipulation and transfer to seawater, one 4º and one 15 ºC tank were gradually acclimatized, and together with one of the 8 ºC tanks adjoined into a separate tank for waterborne *M. viscosa* challenge. In parallel, the fish in the equally acclimatised remaining tanks were sampled for length and weight measurement, vaccination side-effect assessments, and plasma antibody analyses. The outcome of the experimental challenge was assessed after 27 days based on cumulative mortality per group and the presence of skin ulcers consistent with *M. viscosa* infections. No notable difference in growth was found between the vaccination groups at either temperature regime. Abdominal side-effects were all on average below 1.8 on the Speilberg scale in all groups reared at 4°C, but clearly lower (<1.2) in all groups reared at 8ºC or 15°C. The cumulative mortality in unvaccinated controls was close to 60% irrespective of rearing temperature. All vaccination groups showed significant protection, albeit somewhat variable between immunization temperatures. The highest vs. the lowest cumulative mortality of the vaccinated fish varied from 26 vs. 7%, 16 vs. 7% and 22 vs. 7% in the 15, 8 and 4°C immunised groups, respectively. One of the vaccine groups consistently showed the lowest cumulative mortality, indicating the importance of vaccine formulation irrespective of immunization temperature and time. Antibody ELISA outcomes are still being analysed, and correlates of the antibody responses and their association with challenge data and necropsy findings will be presented.

GARLIC AS AN ANTI-PARASITIC THERAPEUTANT IN FISH

J.H. KIM[1], S. FRIDMAN[1], H. BOROCHOV-NEORI[2], T. SINAI[1], D. ZILBERG[1]*

[1]*The Jacob Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Midreshet Ben Gurion, Israel*
[2]*Southern Arava Research and Development, Hevel Eilot, Israel*

Garlic is a broad-spectrum, antimicrobial agent, exhibiting antibacterial, antifungal, antiprotozoal and antiviral properties, which, historically, has been used as a medicinal remedy to treat a wide range of conditions (Ankri and Mirelman, 1999). The current work aimed to test the potential of garlic as a treatment against protozoan parasites of fish.

Two protozoan parasites of teleosts, *Cryptocaryon irritans* and *Tetrahymena* sp., were selected as a model for this study. *In vitro* analysis of parasites' survival following exposure to aqueous garlic extract was carried out by direct microscopic observation and colorimetrically by analyzing mitochondrial activity (MTT assay; Zilberg and Sinai, 2006). Results revealed a clear anti-parasitic activity and a dose-related effect. Garlic inhibited mitochondrial activity in *Tetrahymena* at 40 ppt (19.2 µg of allicin/ml) and immobilized *C. irritans* theronts and trophonts at 5 ppt (2.05 µg of allicin/ml).

To examine the effects of garlic as an oral treatment against *C. irritans*, feeding trials were carried out. In three separate feeding trials, guppies were fed 5%, 10% and 20% of garlic-supplemented food for 2 or 4 weeks (2 % of BW per day in 2 separate feedings), and non-supplemented food as a control. At the end of the feeding, fish were challenged with *C. irritans* and infection levels were examined at 3 days post-challenge. Results suggest that garlic-supplemented food is not effective as a treatment against *C. irritans*. Analysis of immune parameters and histopathology of the differently fed fish is underway.

To examine the effect of storage, freeze-dried powder and aqueous extract of garlic were stored at -20°C, room temperature, 40°C and 60°C, followed by anti-bacterial test against *Vibrio anguillarum* and a chemical analysis of allicin. Anti-bacterial activity in stored garlic extract was reduced over time, *i.e.* 45 % reduction within 2 weeks at room temperature, 50% reduction over 24 h at 40°C and no activity after 48 h at this temperature. Exposure to 60°C resulted in no anti-bacterial activity within 24 h. The freeze-dried powder appeared much more stable, its anti-bacterial activity gradually reduced over time, but the maximal reduction over 8 weeks at 60°C was only 25 %.

AMOEBIC GILL DISEASE IN NORWEGIAN ATLANTIC SALMON (*SALMO SALAR L.*) – CASE HISTORY AND COMPARISON OF TREATMENT WITH H_2O_2 AND FRESHWATER

D. PERSSON[1], T. M. PEDERSEN[1]*, S. KOLSTØ[1], H. HELLBERG[2]

[1]*FoMAS – Fish health service, Haugesund, Norway*
[2]*Fish Vet Group Norge, Oslo, Norway*

The autumn of 2014 was the second year with major outbreaks of amoebic gill disease (AGD) caused by the amoeba *Paramoeba perurans* along the Norwegian coastline. The disease has been diagnosed and treated as far north as the county of Sør-Trøndelag. Treatment options are freshwater in wellboat or hydrogen peroxide (H_2O_2) in wellboat or tarpaulins. One farm was followed closely through two treatments against AGD in the autumn of 2014, with tissue sampling and continuous assessment of gross gill score to investigate possible differences in gill pathology and treatment efficacy.
AGD was diagnosed early in September 2014 and was treated with H_2O_2 in wellboat 2 weeks later. There was a reduction in "active" gill score post treatment, however amoebas were still detected. Comparison of histology sections prior and post treatment, showed amoebas to be present before treatment and considerably reduced after (a few amoebas were detected in one gill post treatment). In addition, mild histopathological lesions associated with H_2O_2 were detected post treatment. "Total" and "active" gill score increased during the next weeks post treatment. 3 weeks after the first treatment, the "active" gill score had doubled compared to 1 week post treatment, and large amount of amoebas were detected in fresh gill smears. A second treatment was performed, 4 weeks after the first one, this time freshwater was used in some cages. For both treatments, Ct-values from qPCR samples indicated a large reduction of amoebas 1 week post treatment, and no amoebas was detected in fresh gill smears. "Active" gill score was reduced, and 1 week post treatment it was lower than at the start of the outbreak in September. Histology showed typical AGD lesions in all fish before and after treatment, but amoeba were not detected post treatment. However, in gills from H_2O_2-treated fish, acute epithelial lesions with hypertrophy and "lifting" were observed. Similar acute lesions were not observed in gills from freshwater treated fish.

MOLECULAR TRACING OF VHS IN DENMARK

S.S. MIKKELSEN[1]*, H. SCHUETZE[2], H. KORSHOLM[3], B.B. JENSEN[4], M.S. BRUUN[1], N.J. OLESEN[1]

[1]*National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark*
[2]*Friedrich-Loeffler Institut, Insel Riems, Germany*
[3]*Danish Veterinary and Food Administration, Vejle, Denmark*
[4]*Norwegian Veterinary Institute, Oslo, Norway*

MOLTRAQ is a pan-European project that aims to increase knowledge on a wide array of economically important viral diseases in fish and molluscs on both the epidemiological and the genetic level. It centers on the use of spatio-temporal and phylogenetic information to create phylogeographic and scenario-simulation models to identify important factors for the spread of disease and to develop and evaluate new control strategies.
Viral haemorrhagic septicaemia Virus (VHSV) is one of the most important viral fish diseases and is widely spread all over Europe and creates significant losses every year for European fish farmers. VHSV has been endemic in Denmark since the 1950's but after an effective control and eradication programme that spanned more than 45 years the virus was finally eradicated from Denmark in 2009.
As part of MOLTRAQ more than 200 Danish isolates, including isolates from both marine and freshwater outbreaks, spanning from 1978-2003 were selected for analysis. The full-length G-gene was sequenced for all isolates and together with epidemiological information these data are being used to create phylogenetic and phylogeographic models to help infer the relationship between VHS outbreaks in Denmark and to look into the spread of the disease over a historical period as well as the effectiveness of containment and eradication programmes.
Molecular tracing shows that the numerous VHS outbreaks in marine fish farms were due to stocking these with VHS infected rainbow trout in the incubation phase and not to infection with VHSV from the marine environment. From evaluating more than 400 VHSV isolates from Denmark it appears that evolution of low virulent VHSV from marine fish species is a very rare event and is most likely related to feeding with fresh fish, which is now prohibited in rainbow trout farming.

MOLTRAQ is funded under the EMIDA-ERA Net under the EU 7th Framework program.

Partners into the project are: Norwegian Veterinary Institute (NO, Coordinator), Technical University of Denmark-National Veterinary Institute (DK), Agence Nationale de Sécurité Sanitaire (FR), Friedrich-Loeffler Institut (DE), Institut Francais de Recherche pour l'Exploitation de la Mer (FR), Institut de Recherche pour le Développement (FR) and Norwegian Computing Center (NO).

COURSE OF INFECTION WITH LYMPHOCYSTIS DISEASE VIRUS IN GILTHEAD SEABREAM (*SPARUS AURATA*)

E.J. VALVERDE[1]*, J.B. ORTIZ-DELGADO[2], M.C. SARASQUETE[2], J.J. BORREGO[1], D. CASTRO[1]

[1]*Universidad de Málaga, Málaga, Spain*
[2]*Instituto de Ciencias Marinas de Andalucía (ICMAN, CSIC), Puerto Real (Cádiz), Spain*

Lymphocystis disease virus (LCDV) is the etiological agent of lymphocystis disease (LCD), a pathology that affects a wide variety of fish species. Data about LCDV pathogenesis are very short, and mainly limited to histopathological studies of skin lesions. Recent studies on viral genome detection (both by PCR or DNA-DNA *in situ* hybridization) suggest that LCDV establish a systemic and persistent infection in gilthead seabream (*Sparus aurata*), but further studies are necessary to prove if this infection is productive or not.
In the present study viral quantification and viral mRNA detection (by qPCR and RT-qPCR) have been used to investigate LCDV multiplication in different organs of juvenile gilthead seabream. In addition, a histopathological study was carried out. Animals were collected from two commercial farms in Southwestern Spain. In one farm, where no LCD outbreaks have been recorded, apparently healthy fish were collected, whereas in the other farm, diseased and recovered (two months after LCD symptoms disappearance) fish were sampled. All the animals were LCDV-infected, and viral gene expression was detected in every organ analysed (caudal fin, intestine, liver, spleen, kidney and brain). In asymptomatic animals, both apparently healthy and recovered, a low-titre infection was observed, with the highest viral copy numbers detected in brain and kidney. In diseased fish, viral loads were significantly higher than in subclinical infected animals, being maximal in caudal fin, where lymphocysts were present in the dermis. Different histological alterations were observed in the internal organs from diseased fish analysed, although no hypertrophied cells were detected in any of them. In recovered fish, most of the organs examined presented similar histological features to those in healthy animals. Thus, pathological changes were only detected in the intestine and liver, although they were less severe than those observed in diseased fish. The results presented showed that LCDV establishes a systemic infection in juvenile gilthead seabream, which can be subclinical. In addition, although the disease is self-limiting, the virus is not removed after disease recovery, but produces a persistent infection.

This study has been supported by projects AGL2010-17880 (Spanish Government, co-funded by the FEDER), and P12-RNM-2261 (Junta de Andalucía).

VIRULENCE ACQUISITION IN INFECTIOUS SALMON ANAEMIA VIRUS: THE ROLE OF MUTATIONS IN THE HAEMAGGLUTININ-ESTERASE AND FUSION PROTEIN

M. FOURRIER*, K. LESTER, A. MCBEATH, B. COLLET

Marine Scotland Science, Marine Laboratory, Aberdeen, UK

Infectious Salmon Anaemia Virus (ISAV) is an orthomyxovirus causing disease in farmed Atlantic salmon. It is an enveloped virus with a genome consisting of eight negative sense single-stranded RNA segments. Segments 6 and 5 encode the major surface glycoproteins Haemagglutinin-Esterase (HE) and the Fusion (F) protein, respectively. HE is the receptor binding protein while the F protein is responsible for the fusion of the viral and host cell membranes during the early stages of infection.

Since the discovery of an HPR0 avirulent variant and the association of deletions in the segment 6 Highly Polymorphic Region (HPR) with pathogenic strains, there has been a strong suspicion that ISAV surface glycoproteins play an important role in determining virulence. However, functional analyses of ISAV surface glycoproteins are lacking which has hindered our ability to understand the mechanisms leading to virulence acquisition.

We recently performed two extensive studies using point mutations on both the HE and F proteins from HPR0 wild types and fusion assays based on ghost erythrocyte techniques. This allowed us to investigate potential virulence markers and understand the role of the segment 6 HPR deletions and the combined effect of segment 5 and 6 mutations during fusion.

Results indicated that mutations on either of these surface glycoproteins can have a profound effect on viral fusion and influence specific stages of this process, such as activation or proteolytic cleavage of the F protein.

These findings shed some light on how the combined effect of mutations in the HE and F proteins may determine virulence in ISAV. This mirrors some of the mechanisms previously reported in influenza and Newcastle Disease Virus.

These results also emphasise that HE HPR deletions alone may not provide a full picture of an isolate virulence. The F protein composition, structure and how it interacts with the HE are also key determinants of viral fusion and virulence.

FIRST ISOLATION OF A RHABDOVIRUS FROM PERCH IN SWITZERLAND

T. WAHLI[1]*, L. BELLEC[2], B. VON SIEBENTHAL[1], J. CABON[2], H. SCHMIDT-POSTHAUS[1], T. MORIN[2]

[1]*Centre for Fish and Wildlife Health, Bern, Switzerland*
[2]*French Agency for Food, Environmental and Occupational Health & Safety, European University of Brittany, Plouzané, France*

Rhabdoviruses infect a wide range of hosts, including vertebrates, invertebrates and plants. They are among the most devastating viruses for the aquaculture worldwide and, under certain circumstances, they can cause a significant ecological impact on wild fish populations.
Perca fluviatilis is a fish species of increasing interest for the Swiss fish farming industry and recirculation systems have been specifically set up in recent years to develop its production. In one of these farms, an aberrant spiraling swimming associated to elevated mortalities occurred repeatedly in imported fish shortly after stocking. No bacterial or parasitic etiology was detected but a Perch rhabdovirus was isolated on BF-2 cells and identified using a specific indirect fluorescent antibody technique (IFAT). Subsequent investigations on other samples suggested a special viral tropism for the central nervous system (CNS). Phylogenetic analysis on the partial N and entire G gene sequences positioned this Swiss isolate in the genogroup C of the Perch rhabdovirus species, with high nucleotide and amino acid (aa) identities with the DK5533 strain isolated in Denmark in 1989. Comparative studies using other isolates allowed to distinguish two serological patterns among Perch rhabdoviruses and to identify an aa position in the glycoprotein potentially involved in the antigenic differentiation. Even if perch recently imported in the farm were tested negative in virology prior to transport, they may have been the origin of this outbreak because CNS was not included in the samples analyzed. Another possibility might be a covert infection in the farm with a virus load in resident fish too low to be detected. This study reports the first case of Perch rhabdovirus disease in a Swiss farm and underlines the difficulty to effectively diagnose infection in asymptomatic fish. The high identity of the Swiss Perch rhabdovirus isolate with a strain described in Denmark in 1989 strongly suggests that movements of fish contribute to the spread of pathogens, particularly for species in development for which animal science and knowledge of infectious diseases are to deepen.

MOLECULAR DETECTION AND GENOME ANALYSIS OF CIRCOVIRUSES OF EUROPEAN EEL *(ANGUILLA ANGUILLA)* AND SICHEL (*PELECUS CULTRATUS)* FROM LAKE BALATON, HUNGARY

R. BORZÁK*, B. SELLYEI, CS. SZÉKELY, A. DOSZPOLY

Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

The effect of the climate change on pathogen distribution is being examined in the framework of a national monitoring program 'Bioclimate' in Hungary. In the project freshwater fishes were screened for the presence of circoviruses, as well.
Circoviruses are small, non-enveloped viruses with a circular single-stranded (ss) DNA genome ranges about 1.3-2.3 kb in size. The genome contains at least two open reading frames (ORF), positioning in opposite directions and encoded replication (Rep) and capsid (Cap) proteins.
For the detection of circoviruses a broad-spectrum nested PCR was used targeting a conserved region of the Rep gene. In case of positive results inverse nested PCR or RCA were carried out to amplify the remaining part of the circular genomes.
Surprisingly, high prevalence of circoviral DNA was detected in the European eel (*Anguilla anguilla*) population in Lake Balaton. Nineteen out of 31 eels proved to be positive by PCR. Comparing the partial rep-like sequences, 11 of them have identical nucleotide sequences with the previously described European eel circovirus (EeCV) (Doszpoly et al., 2014). Eight samples were similar to the above-mentioned ones with 96% nucleotide identity and were identical to the rep-like sequence originating from a sichel (*Pelecus cultratus*) caught also in Lake Balaton.
Until now, the complete genome sequencing of 11 eel and one sichel circoviruses were completed successfully. Ten of the newly sequenced eel circovirus genomes were identical with the formerly reported EeCV genome while the remaining eel circovirus and the sichel circovirus genomes showed 100% nucleotide similarity to each other.
Circoviruses were described as host-specific or narrow host range microorganisms. To the best of our knowledge, this is the first report about the detection of very similar circovirus related sequences (probably the same virus species) in various, distantly related fish species.

This study was supported by KTIA-AIK-12-1-2013-0017 and OTKA PD104315 grants and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

DETECTION AND TISSUE DISTRIBUTION OF OSTREID HERPESVIRUS 1 PROTEINS IN INFECTED PACIFIC OYSTER, *CRASSOSTREA GIGAS*

C. MARTENOT[1]*, A. SEGARRA[1], L. BAILLON[1], N. FAURY[1], M. HOUSSIN3, T. RENAULT[2]

[1]*Ifremer (I*nstitut Français de Recherche pour l'Exploitation de la Mer) Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France
[2]*Ifremer* Departement Ressources Biologiques et Environnement, Nantes, France
[3]*LABEO Frank Duncombe, Caen, France*

Ostreid herpesvirus 1 (OsHV-1) is a major threat to oyster cultivation in Europe since several decades and is associated with mass mortality events affecting the early-life-stage of Pacific oyster, *Crassostrea gigas*.
An immunohistochemistry (IHC) protocol for detecting three OsHV-1 proteins (two putative membrane proteins and one putative apoptosis inhibitor protein) was used to describe the viral distribution in experimentally infected oysters. A viral suspension was injected in the adductor muscle of spat before to be sampled at 1h30, 5h30, and 27h30 post-injection. Polyclonal antibodies against two putative membrane proteins and one putative apoptosis inhibitor protein were produced in rabbit to target OsHV-1 proteins with DAB chromogenic detection.
A nuclear localization of the putative apoptosis inhibitor protein was observed in connective tissue of various organs such as gills, mantle, adductor muscle, digestive gland, heart, and labial palps. This distribution is in accordance with the scientific literature including the detection of viral particles by transmission electron microscopy in infected oysters. Additionally there was a positive signal in the nucleus and the cytoplasm of some ovocytes and labeled cells were also detected in the epithelium of some digestive tubules. A cytoplasmic localization of the two putative membrane proteins was detected in the epithelium of gills, mantle, digestive gland, labial palps and also in the connective tissue of gills and labial palps. At 1h30 post-injection, OsHV-1 proteins were located in the majority of infected oyster tissue whereas the percentage of labeled cells was the most important at 27h30 post-injection for the putative apoptosis inhibitor protein. These results were in accordance with those obtained using *in situ* hybridization (DNA probe) performed on the same animals. Moreover, abnormal chromatin patterns in cells infected oysters were observed in association with the detection of OsHV-1 proteins. However, few positive signals were observed in healthy appearing oysters and could be due to virus persistence after a primary infection. In conclusion, IHC could be an interesting method for analyzing the early infection stages of OsHV-1 infection and a useful tool to investigate protein interactions between host and OsHV-1.

This work received financial support from the European project "MOLecular TRacing of viral pathogens in aQuaculture" (MOLTRAQ).

ISOLATION AND CHARACTERIZATION OF *PSEUDOMONAS ANGUILLISEPTICA*-LYTIC PHAGES BELONGING TO *MYOVIRIDAE*, *SIPHOVIRIDAE* AND *CYSTOVIRIDAE*

I. ISTIQOMAH[1]*, R. MATSUMOTO[1], Y. KAWATO[2], Y. SHIMAHARA[2], T. NAKAI[1]**
***corresponding author*

[1]*Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan*
[2]*National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Japan*

Pseudomonas angilliseptica was first described as the causative agent of "red spot disease" in farmed Japanese eel *Anguilla japonica* and European eel *A. anguilla*. Thereafter, the disease was recorded in various non-angillid fish species for aquaculture in many European and East Asian countries. *P. anguilliseptica* strains are rather uniform in the biochemical and physiological characteristics, but serological analyses divided them into two K (heat-labile antigen) serotypes and two O (heat-stable antigen) serotypes, which are correlated well with the host-specificity or virulence of the pathogen. For further characterization of *P. anguilliseptica* and establishment of the method to control the disease, we isolated *P. anguilliseptica*-lytic phages from various aquaculture environments in different areas of Japan. A total of 15 lytic phages were isolated by an enrichment and double-layer agar methods. Based on the morphological and nucleic acid features, they were identified as contractile tailed-*Myoviridae* (n=8, dsDNA, 4 RFLP types), non-contractile long tailed-*Siphoviridae* (n=4, dsDNA, 3 RFLP types) and polyhedral enveloped-*Cystoviridae* (n=3, dsRNA). Infectivity tests of the phages against 30 *P. anguilliseptica* isolates including type strain (NCMB1950), which had been isolated from diseased Japanese eel, ayu *Plecoglossus altivelis*, Japanese flounder *Paralichthys olivaceus*, black scraper *Thamnaconus modestus*, striped jack *Pseudocaranx dentex* and red sea bream *Pagrus major* in Japan, resulted in 11 infective types. Myoviruses showed the most broad infectivity with some exceptions, while both shiphoviruses and cystoviruses are particularly infective to *P. anguilliseptica* isolates from Japanese eel. The present phages will be useful for comparing surface components (phage receptors) among *P. anguilliseptica* strains.

A NETWORK FOR IMPROVEMENT OF CEPHALOPOD WELFARE AND HUSBANDRY IN RESEARCH, AQUACULTURE AND FISHERIES - COST ACTION FA1301, CEPHS*IN*ACTION

G. PONTE[1, 2]*, C. GESTAL[3], S. CULLOTY[4], D. OSORIO[5]

[1]*Cephalopod Research – CephRes, Napoli, Italy*
[2]*Presentation on behalf of 43 Members of the Management Committee of CephsInAction*
[3]*Institute of Marine Research (IIM-CSIC), Vigo, Spain*
[4]*University College Cork, Cork, Ireland*
[5]*University of Sussex, Brighton, UK*

Cephalopod mollusks are the sole invertebrates included in the European Directive on protection of animals used for scientific purposes (Directive 2010/63/EU). The Directive gives special attention to the improvement of animal welfare, and adheres closely to the principle of the 3R's (Reduction, Refinement and Replacement). Among the about 700 living cephalopod species, only 20 are currently utilized as "Laboratory Animals", and few others are exploited for their potential in aquaculture. Research on cephalopods can be dated back to the XIX century. The scientific interest for these species has been always mixed with a social dimension, including being part of our diet or as characters in arts, literature, and advertising. The richness of their behavioral repertoire, and the diversity of forms and physiological adaptations represent a challenge for the scientific community. Compliance with the Directive 2010/63/EU and the increased concern about welfare issues faced scientists and regulators an added challenge. Cephs*In*Action has the ambitious aim of addressing this challenge. As a COST Action, itis able to foster an interdisciplinary network of experts aiming to promote the exchange of tools, training, and to increase scientific knowledge with the overall objective of establishing sound systems for the care of cephalopods in different contexts. Cephs*In*Action operates to facilitate multi-disciplinary and inter-species scientific exchanges to integrate knowledge on welfare practices, and to promote cephalopod research, conservation and public awareness.
Activities of Cephs*In*Action are organzed into five WGs with the task of: *i*. improve knowledge on the care and welfare of animals to facilitate standardization of procedures among different laboratories, *ii.* develop new approaches to promote 3Rs principle and application of alternative methods, *iii.* disseminate results and knowledge available and promote training and education.
Particularly WG2 is focused on the: *i.* evaluation of signs and indicators of stress, pain, suffering and immune response, *ii.* classification, diagnosis and prevention of diseases in cephalopods, *iii.* exploration of non-inavasive methods to assess the health conditions in live individuals.
Cephs*In*Action (www.cephsinaction.org) includes 18 countries, and involves more than 150 researchers.

This presentation is considered as contribution to the activities of the COST Action FA1301 - CephsInAction.

PARASITES AND DISEASES OF WORLDWIDE CEPHALOPODS: AN UPDATE

P. TEDESCO[1, 2]*, A. TERLIZZI[1], G. FIORITO[3]

[1]*Università del Salento, Lecce, Italy*
[2]*Association for Cephalopod Research-CephRes, Italy*
[3]*Stazione Zoologica Anton Dohrn, Naples, Italy*

Cephalopods are known to harbor parasitic organisms in almost every organ of their body. The growing importance of cephalopods for both commercial and research purposes requires a better understanding about their health and welfare, which includes a detailed characterization of their parasites and diseases, especially of those infectious agents that are able to affect the physiology of cephalopod hosts or their marketability as fishery products. Information about the parasitic fauna of cephalopods also prove very useful for scientists since it may help elucidating some aspects of cephalopod biology and ecology in natural environment.
During the last decades, the development of new investigation techniques, such as the increased use of molecular tools, allowed the identification of new parasite species and helped to solve the taxonomic uncertainty resulting from difficulties associated with morphological identification of small-sized and often cryptic organisms, which had produced, over time, a high synonymy rate among parasitic taxa. The present work intends provide an update of the last 25 years of research about cephalopod infectious agents, by considering over two hundred new publications, since they were first extensively surveyed in 1990. The number of new parasite descriptions, new host records and new infection reports was reviewed as well as the geographic distribution of reports in order to characterize the worldwide pattern of research efforts in cephalopod parasites.
The total amount of literature available so far was also considered and analyzed in order to detect potential variations in parasite biodiversity among cephalopod groups as well as among geographic areas and to identify parasitic groups, host groups and geographic areas that require more investigation. Special attention is paid to potentially pathogenetic agents and to treatments available or attempted so far.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

IMPLEMENTATION OF THE DISEASE DATABASE FOR CEPHALOPODS

V. GALLIGIONI[1]*, C. GESTAL[2], P.TEDESCO[3, 4], G. FIORITO[3]

[1]*FNUSA-ICRC, Brno, Czech Republic*
[2]*IIM-CSIC, Vigo, Spain*
[3]*Stazione Zoologica Anton Dohrn, Naples, Italy*
[4]*Universita' del Salento, Lecce, Italy*

Cephalopods have been included in the European Directive (EU Directive 63/2010), that rules the use of animals for scientific purposes. Therefore, animals welfare and health should be assured. Considering cephalopods, only few information are available on their microbiological status, microbiological agents affecting them and the micro-organism host interaction. The Working Group 'Stress and diseases' of the COST Action FA1301, CephsInAction, is aimed to classify diseases of cephalopods and establish guidelines for diseases prevention.
Diseases can affect animals on different levels: they could show clinical signs (lesions, ulcer, bleeding), behavioural abnormalities (i.e. lethargy, being not attracted by the prey, inking) or can not respond as usual to normal stimuli. Therefore, experiments can be not reproducible, data can be highly affected and the research can be not robust at all. Nowadays, veterinarians, researchers and technical personnel involved in cephalopods projects don't have many available tools for monitoring the health status and there are only few qualified experts to evaluate the cephalopods well-being.
For this reason, we have collected all papers available on clinical reports and experimental infections and we have analysed them for all scientific information reported. Based on these annotations, we propose a cephalopods diseases database, including pathogens, hosts, symptoms, tissues affected, diagnostic methods, survival rate and possible treatment. Filters for pathogens or for symptoms can be applied. Moreover, the database is correlated to one atlas corresponding to lesions and symptoms.
Animal care-takers, researchers running experiments and technician will have the tool to discriminate lesions (physiology vs pathology), to recognize and study the pathogen, to reduce the spread of infection and to find possible treatment. Moreover, the personnel responsible for the animal welfare, designated veterinarians and officers will have one possible tool to check if animals are kept in a good health status and according to the authorized experimental project.
In the future, this database will be continuously updated with case-reports and to make it available to researchers, designated veterinarians and officers.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

SELECTION OF RELIABLE REFERENCE GENES FOR NORMALIZATION OF GENE EXPRESSION IN PARALARVAE OF *OCTOPUS VULGARIS* DURING DEVELOPMENT AND AFTER IMMUNE-STIMULATION

P. GARCÍA-FERNÁNDEZ[1]*, S. CASTELLANOS-MARTÍNEZ[1, 3], J. IGLESIAS[2], J.J. OTERO[2], C. GESTAL[1]

[1]*Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas (IIM-CSIC), Vigo, Spain*
[2]*Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Vigo, Spain*
[3]*Centro de Investigación y de Estudios Avanzados del IPN Unidad Mérida, Yucatán, México*

The common octopus, *Octopus vulgaris*, is a new candidate species for aquaculture. However, rearing of octopus paralarvae is hampered by high mortality and poor growth rates which impede its entire culture. The study of genes involved in the octopus ontogenesis and immune response capability could help to understand the key of paralarvae survival and thus, to close the octopus life cycle. Real-time (RT-qPCR) is the most common tool to quantify the gene expression because their specificity and sensitivity. The expression level of mRNA can vary significantly in different developmental stages, tissues or infection conditions. Therefore, the reability of RT-qPCR requires the selection of appropriate normalization genes, whose expression must be stable across the different conditions of each study. Hence, the aim of the present work is to evaluate the stability of eight candidate genes: ß-actin (ACT-1 and ACT-2), elongation factor 1 α (EF-1 and EF-2), ubiquitin (UBI), ß-tubulin (TUB), glyceraldehyde 3-phosphate dehydrogenase (GADPH) and ribosomal RNA (18S) in order to select the best reference gene. The expression analysis was studied using cDNA obtained after RNA extraction from octopus paralarvae of 7 different developmental stages (embryo, paralarvae of 0, 10, 15, 20, 30 and 34 days). In addition, gene expression was analyzed in paralarvae of 22 days immune-stimulated after challenge with Gram-negative pathogenic bacteria *Vibrio lentus* and *V. splendidus*. Gene expression was quantified by RT-qPCR and expression stability was analyzed with three different software applications, *GeNorm*, *NormFinder* and *Bestkeeper*. Our results showed UBI, 18S and EF-1 as the most stable reference genes to use as normalizing genes during ontogeny of octopus paralarvae. For immune-stimulated samples, UBI, ACT-2 and ACT-1 were the most stable genes and therefore the most suitable for RT-qPCR normalization. To additional experimental conditions, the stability of the genes here identified as reference should be tested.

This work was founded by the research project AGL 2013-49101-C2-2-R from the Ministerio de Economía y Competitividad (Spain). P. García-Fernández thanks Xunta de Galicia-CEI Campus do Mar, Universidad de Vigo for his predoctoral contract.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

ULTRASTRUCTURAL AND MOLECULAR CHARACTERIZATION OF *AGGREGATA* SPP (FRENZEL, 1885) IN *OCTOPUS VULGARIS* (CUVIER, 1797) FROM CENTRAL MEDITERRANEAN

P. TEDESCO*[1], C. GESTAL[2], A. TERLIZZI[1], G. FIORITO[3]

[1]*Università del Salento, Lecce, Italy*
[2]*Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Cientificas (IIM – CSIC), Vigo, Spain*
[3]*Stazione Zoologica Anton Dohrn, Naples, Italy*

The work focused on ultrastructural and molecular characterization of protozoan parasites of the genus *Aggregata* (Apicomplexa, Aggregatidae) in specimens of the common octopus *Octopus vulgaris* obtained from Ionian and Tyrrhenian seas (Southern Italy, Central Mediterranean). *Aggregata* spp. are reported to parasitize cephalopod species and to be responsible of severe pathologies, which seem to be mostly evident in cultured cephalopods.
In the Central Mediterranean the parasite is known to occur with high prevalence, however its taxonomic status in this area is still under debate.
Following detection and purification of parasitic stages from octopus tissues, genomic DNA was extracted from *Aggregata* sporocysts. 18S rRNA gene of the parasite was amplified by PCR using specific primers and the sequences obtained were compared to those available from *Aggregata octopiana* type species infecting *O. vulgaris* in Northeastern Atlantic.
Samples for Transmission Electron Microscopy (TEM) were treated using standardized protocols specifically designed for the processing of *Aggregata* spp. parasites. Histological preparations obtained from samples of infected oesphagus, crop, caecum, intestine and mantle of *O. vulgaris* showed the presence of both gamogonic and sporogonic stages of *Aggregata* and allowed the use of a semiquantitative method to compare the intensity of infection per each sampled locality. Information from histology was integrated with the analysis of parasite ultrastructure by TEM, which allowed the observation of important taxonomic features to distinguish between different species of *Aggregata*.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

RECENT ADVANCES AT FUNCTIONAL AND MOLECULAR LEVEL OF THE INTERACTION BETWEEN THE CELLULAR INNATE IMMUNITY OF THE COMMON OCTOPUS, *OCTOPUS VULGARIS,* AND THE COCCIDIA, *AGGREGATA OCTOPIANA*

S. CASTELLANOS-MARTINEZ*, C. GESTAL

Institute of Marine Research (IIM-CSIC), Vigo, Spain

Octopus vulgaris is a cephalopod of high economic importance successfully reared in Spain. However, the octopus well-being is threatened by the gastrointestinal coccidian *Aggregata octopiana*. In order to control and eradicate diseases of octopus aquaculture, studies focused on knowing the octopus immune response against pathogens are needed. The present work exposes the advances reached on functional and molecular aspects towards to elucidate the crosstalk between the octopus immune system and the coccidian parasite. Studies conducted through light and electron microscopy, and flow cytometry allowed the characterization of two sub-populations of hemocytes: large and small granulocytes that differ in size and granularity. Large granulocytes showed higher ability to engulf particles and produce respiratory burst (ROS) than small granulocytes. The Nitric Oxide (NO) production, measured in the total population of hemocytes, was higher after challenging the cells with zymosan than LPS or PMA after 3 h of incubation. The assessment of the effect of the *A. octopiana* infection intensity by functional hemocytic assays showed that coccidiosis induced an increase of phagocytosis ($P< 0.05$), but decrease of ROS ($P<0.01$) and NO ($P<0.1$). *De novo* transcriptomic sequencing of octopus hemocytes using the high-throughput Illumina technology allowed the identification of a repertory of genes involved in immune pathways such as complement, TLR, NFκB and apoptosis. Moreover, 539 genes were differentially expressed between sick and healthy octopuses affected by *A. octopiana*. A RT-qPCR confirmed the expression pattern of genes involved in octopus immune response with the high-throughput results. Complementarily, the proteomic analysis of hemocytes allowed the identification of 42 significant proteins differentially expressed between sick and healthy octopuses. According to PCA, 7 of these proteins are potential candidate of resistance biomarkers against the coccidiosis. Fascin and filamin were up-regulated in the proteome of sick octopuses, corresponding to a high phagocytic capability. Contrarily, peroxiredoxin, an antioxidant protein that protects the hosts against oxidative stress, was down-regulated in the same octopus group and agree with a low ROS production. In summary, functional and molecular data suggest that *A. octopiana* might be involved in immunomodulation of the octopus immunity. The first molecular insights of this relationship are here provided.

CONTROLLED NOISE EXPOSURE EXPERIMENTS ON CEPHALOPODS

M. SOLÉ[1]*, M. LENOIR[2], M. DURFORT[3], M. LÓPEZ-BEJAR[4], A. LOMBARTE[5], M. ANDRÉ[1]

[1]*Laboratory of Applied Bioacoustics, Technical University of Catalonia, Barcelona, Spain*
[2]*INSERM U.1051, Institute of Neurosciences of Montpellier, Montpellier, France*
[3]*Department of Cellular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain*
[4]*Department of Animal Health and Anatomy, Faculty of Veterinary Science, Universitat Autònoma de Barcelona, Cerdanyola del Vallés, Spain*
[5]*Renewable Marine Resources Department, Marine Sciences Institute (ICM-CMIMA-CSIC), Barcelona, Spain*

Many anthropogenic noise sources are nowadays contributing to the general noise budget of the oceans. The extent to which sound in the sea impacts and affects marine life is a topic of considerable current interest both to the scientific community and to the general public. Cepaholopods potentially represent a group of species whose ecology may be influenced by artificial noise that would have a direct consequence on the functionality and sensitivity of their sensory organs, the statocysts. These are responsible for their equilibrium and movements in the water column. Controlled Exposure Experiments, including the use of a 50-400Hz sweep (RL = 157±5 dB re 1 µPa with peak levels up to SPL = 175 dB re 1 µPa) revealed lesions in the statocysts of four cephalopod species of the Mediterranean Sea, when exposed to low frequency sounds: (n=76) of *Sepia officinalis,* (n=4) *Octopus vulgaris,* (n=5) *Loligo vulgaris* and (n=2) *Illex condietii.* The analysis was performed through scanning (SEM) and transmision (TEM) electron microscopical techniques of the whole inner structure of the cephalopods' statocyst, especially on the *macula* and *crista*. All exposed individuals presented the same lesions and the same incremental effects over time, consistent with a massive acoustic trauma observed in other species that have been exposed to much higher intensities of sound: Immediately after exposure, the damage was observed in the *macula statica princeps* (*msp*) and in the *crista* sensory epithelium. Kinocilia on hair cells were either missing or were bent or flaccid. A number of hair cells showed protruding apical poles and ruptured lateral plasma membranes, most probably resulting from the extrusion of cytoplasmic material. Hair cells were also partially ejected from the sensory epithelium, and spherical holes corresponding to missing hair cells were visible in the epithelium. The cytoplasmic content of the damaged hair cells showed obvious changes, including the presence of numerous vacuoles and electron dense inclusions not seen in the control animals. The lesions described here are new to cephalopod pathology. Given that low-frequency noise levels in the ocean are increasing (e.g. shipping, offshore industry, and naval maneuvers), that the role of cephalopods in marine ecosystems is only now beginning to be understood, and that reliable bioacoustic data on invertebrates are scarce, the present study and future investigations will bring an important contribution to the sustainable use of the marine environment.

This presentation is considered as contribution to the activities of the COST Action FA1301 - CephsInAction.

OCTOPUS VULGARIS IMMUNE RESPONSE AT GENE EXPRESSION LEVEL BY *IN VITRO* IMMUNOSTIMULATION OF HEMOCYTES

M. PETRIĆ[1, 2]*, E. BALDASCINO[2], C. LAURITANO[2], I. ZARRELLA[2], G. PONTE[2], G. FIORITO[2]

[1]*Department of Marine Studies, University of Split, Split, Croatia*
[2]*Stazione Zoologica Anton Dohrn, Napoli, Italy*

Investigations of the cephalopod immune system have recently become of great importance due to the increasing interest for aquaculture, for the role of potential pathogens on the quality of the "sea-products" for human consumption and for concern about cephalopod welfare since the inclusion of this taxon in the Directive 2010/63/EU on the protection of animals utilized for scientific purposes. Cephalopods rely solely on innate immunity and hemocytes are suggested to play a key role in the immune-system response to challenges (e.g. through phagocytosis, encapsulation, infiltration of pathogens).
Aim of this research was to study the elements of cephalopod immune response at gene expression level by in vitro immunostimulation of *Octopus vulgaris* hemocytes. Hemolymph cell suspension was plated with Squid Ringer's Solution and kept at 18 °C prior immune-stimulation with commercially available immunostimulants: LPS (*Escherichia coli* lipopolysaccharides) as bacterial imitator, Poly I:C (polyinosinic-polycytidylic acid sodium salt) as viral imitator and zymosan, a yeast cell extract eliciting fungal type infection response. As control, hemocytes were kept under the same conditions as the immunostimulated ones. Immunostimulation was accomplished within different time-points: 1, 4, 24 and 48 hours. At the end of each time-point hemolymph suspension was collected and centrifuged following pellet resuspension in TriReagent. Total RNA was extracted from hemocytes following manufacturer's protocol (InVitrogen) and RNA samples were retrotranscripted into cDNA, which was utilized as template for PCR and RT-qPCR analyses.
The expression levels of specific gene of interest, immune-related genes and genes associated to the antioxidant system, stress and detoxification, was analyzed. In general, for all the treatments (LPS, Poly I:C and zymosan) and the time points (1, 4, 24 and 48 h) the majority of investigated antioxidant genes (sod, gpx and gsh-s) were up-regulated after 1 and 4 h post-stimulation, and the majority of the investigated immune genes (nfkb, ser and tnf) appeared up-regulated only after 24 or 48 h post-stimulation.

This presentation is considered as contribution to the activities of the COST Action FA1301 - CephsInAction.

WITHDRAWN O-201

THERMOLICER® – A NEW NON-MEDICAL TECHNOLOGY AGAINST SALMON LOUSE (LEPEOPHTHEIRUS SALMONIS) – DOCUMENTATION OF EFFECT AND WELFARE

R.N. GRØNTVEDT[1]*, H. VILJUGREIN[1] A.G. GJEVRE[1], I.K. NERBØVIK[2], A. LITLABØ[3], S. NYGAARD[4]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*Bremnes Seashore AS, Bremnes, Norway*
[3]*Kobbevik og Furuholmen oppdrett, Storebø, Norway*
[4]*Fomas – Fiskehelse og Miljø, Lundegrend, Norway*

In light of resistance development against the different antiparasitic drugs, non-medical methods to control salmon lice in salmon farming has been developed. The Thermolicer® is a delousing machine where the salmon are exposed to temperate seawater (30-33.5°C) for about 25-30 seconds. The treatment detaches salmon lice from the salmon, and lice are subsequently collected in filter systems. In order to evaluate the treatment effect on salmon lice and evaluate the welfare of the fish, a documentation project was conducted. The project was performed in cooperation with five different salmon producers and their fish health services. Two different Thermolicer® machines were used in commercial delousing and sea lice numbers and welfare data were registered from two weeks before delousing to three weeks after delousing. Welfare data included feeding data, mortality numbers and screening of 40 fish before, after and up to three weeks after delousing, where observations of certain external acute injuries were categorized. Statistical analysis of pre- and post-treatment salmon lice numbers show significant effects on mobile- and adult female lice, but not conclusive effects on chalimus stages.
During the project period, the machines (including pumping systems) were continuously optimized, and welfare data was improved during this period. The results from the project indicate that Thermolicer® is a new non-medical technology that significantly reduces sea lice numbers under acceptable welfare conditions for the salmon.

OPTIMIZATION OF THE COHABITATION CHALLENGE MODEL FOR INFECTIOUS PANCREATIC NECROSIS VIRUS IN ATLANTIC SALMON (*SALMO SALAR* L) USING A SYSTEMATIC APPROACH

H.M. MUNANG'ANDU[1]*, N. SANTI[2], B. NILSEN FREDRIKSEN[3], K.-E. LØKLING[3], Ø. EVENSEN[1]

[1]*Norwegian University of Life Sciences, Oslo, Norway*
[2]*AquaGen, Trondheim, Norway*
[3]*Pharmaq AS, Oslo, Norway*

A cohabitation challenge model was developed for use in evaluating the efficacy of vaccines developed against infectious pancreatic necrosis virus (IPNV) in Atlantic salmon (*Salmo salar* L) using a stepwise approach. The study involved identifying a set of input variables that were optimized before inclusion in the model, which included the highly virulent Norwegian Sp strain NVI015TA encoding the $T_{217}A_{221}$ motif having the ability to cause >90% mortality for use as challenge virus. The challenge dose was estimated at $1x10^7$ $TCID_{50}$/fish while the proportion of virus shedder proportion was estimated at 12.5% of the total biomass per tank. The model was designed based on a three parallel tank system in which the Cox hazard proportional regression model was used to estimate the minimum number of fish required to show statistical differences between the vaccinated and control fish in each tank. All input variables were optimized to generate ≥75% mortality in the unvaccinated control fish in order to attain a high discriminatory capacity (DC) between the vaccinated and control fish as a measure of vaccine efficacy. The model shows the importance of using highly susceptible fish to IPNV infection in order to obtain a higher DC for differentiating vaccine protected fish from the unvaccinated control fish. Once optimized, the model was tested for its reproducibility by generating similar results from three independent challenge trials using the same input variables. Overall, data presented here shows that the cohabitation challenge model developed in this study is reproducible and that it can reliably be used to evaluate the efficacy of IPNV vaccines in Atlantic salmon. We envisage that the approach used here shall open new avenues for developing optimal challenge models for use in evaluating the efficacy of different vaccines used in aquaculture.

THE THREAT OF ILLEGAL FISH MOVEMENTS ON NATIONAL FISH HEALTH STATUS

J. MEWETT*

Fish Health Inspectorate, Cefas, Weymouth, UK

The UK has a high fish health status due to longstanding controls on fish disease and is free of most of the EU listed diseases. This status is threatened by illegal imports to the UK driven by demand for big fish for angling purposes. The UK has a large angling industry with approximately 3 million anglers, most of whom catch and release, generating an estimated £3 billion. Supply of large specimen fish is limited within the UK compared to mainland Europe where diseases such as Spring Viraemia of Carp (SVC) are more widespread. Many of these countries do not control SVC and cannot sign health certification to allow legal trade in these large fish.

A case study including video footage from a seizure of illegally imported fish at a port is discussed along with the implications of disease introduction from illegal imports to the health status of the UK. The discussion highlights how the work carried out on fish diseases in research facilities and diagnostic laboratories is applied to regulate our borders and to protect fish health across the EU.

INTERNAL NEOPLASMS IN KOI (*CYPRINUS CARPIO KOI*), FREQUENCY, CHARACTERISATION AND RISK FACTORS FOR DEVELOPMENT

H. SCHMIDT-POSTHAUS[1]*, R. KNÜSEL[2], T. WAHLI[1], F. OTT KNÜSEL[2]

[1]*Centre for Fish and Wildlife Health, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3001 Bern, Switzerland*
[2]*Fishdoc GmbH, Schaubhus, 6026 Rain, Switzerland*

Fish, like mammals, can be affected by neoplastic proliferations. In Switzerland, koi carp (*Cyprinus carpio koi)* with tumours affecting the internal organs are a common finding. Most koi with internal tumours are euthanized due to the fact that

a) surgical removal of intraperitoneal tumours in koi is rather complex,
b) no chemotherapy for koi is available,
c) re-occurrence of tumours in fish is poorly documented, and
d) neoplastic masses are already quite advanced when tumours are diagnosed.

Preventive measures might be a possibility to reduce the frequency of tumours. However, to establish such measures, knowledge on possible causes for the occurrence of tumours is a prerequisite. To gain more information about the nature of intraperitoneal tumours in koi, a study was initiated. Between 2008 and 2012, koi with abdominal swelling were examined pathologically. All neoplastic lesions found were classified histologically whereby their nature and growth characteristics were documented. Pathological analysis revealed that all internal tumours showed several signs of malignancy, which indicates that these tumours are likely to be fast growing masses. Our results indicate that female koi are more prone to develop internal tumours and that the main organs of origin were the gonads. Within the gonads, the main tissue of origin was the sex cord stromal tissue.

To evaluate possible risk factors for development of internal neoplasms in koi we used an online questionnaire sent to fish keepers with koi affected by internal tumours and to fish keepers who had not previously reported any affected koi. Analysis of the obtained data revealed that tumour occurrence was associated with the location and the volume of the pond, with the frequency of water change, with the origin of the koi, with the numbers of koi kept in one pond and with the use of certain pond disinfection / medication products.

Our results contribute to the identification of possible risk factors, which in turn could help to establish prophylactic measures in order to reduce the occurrence of internal neoplasms in koi in the future.

VIBRIO TAPETIS ISOLATED FROM VESICULAR SKIN LESIONS IN DOVER SOLE (*SOLEA SOLEA*)

A.M. DECLERCQ*, K. CHIERS, M. SOETAERT, A. LASA, J.L. ROMALDE, H. POLET, F. HAESEBROUCK, A. DECOSTERE

Ghent University, Ghent, Belgium
University Santiage de Compostela, Santiago De Compostela, Spain
Institute for Agriculture and Fisheries Research, Ostend, Belgium

Vibrio tapetis is primarily known as the causative agent for brown ring disease in bivalves, although it has been isolated from cultivated fish during mortalities on farms.
Here we describe the first isolation of *V. tapetis* from wild-caught and subsequently captive-held Dover sole (*Solea solea*). Pathological features consisted of multifocal circular greyish-white skin discolourations evolving into vesicular lesions and subsequent ulcerations on the pigmented side. On the non-pigmented side, multiple circular lesions - white at the center and red at the edges - were evident. Upon histological examination, the vesicular lesions presented as dermal fluid filled spaces with collagen necrosis and mixed inflammatory infiltrate. Large numbers of small rod-shaped bacteria, up to 1.5µm in length, were present in the vesicle. A mild bacterial invasion was found in the underlying connective tissue with an interstitial mononuclear infiltration. In the deep skin lesions, there was loss of scales and an abrupt transition between epidermis and ulcerated skin with complete necrosis of dermal connective tissue. This ulceration was well-delineated by granulation tissue infiltrated with large numbers of mononuclear cells and moderate numbers of neutrophils, as well as numerous bacteria with different morphology. The myofibers bordering the ulceration were degenerative and fragmented. In between underlying superficial and deep muscle layers, oedema occurred. Lesions were not observed in samples of liver, spleen and kidney. Serotyping, DNA-DNA hybridization and REP- and ERIC-PCR techniques showed that the retrieved isolates belonged to the species *V. tapetis* displaying a profile similar to the representative strain of genotype/serotype O2 originally isolated from Carpet-shell clam (*Venerupis decussata*) and closely related to isolates obtained from Wedge sole (*Dicologoglossa cuneata*).
In conclusion, this case study is the first report of the isolation of *V. tapetis* from skin vesicles and ulcerations in Dover sole. This agent may have played a role in the development of the skin lesions. However, further studies are needed as any pathological association between the noted lesions and the above agent currently is presumptive.

WITHDRAWN O-206

MOLECULAR CONFIRMATION OF INFECTIOUS SPLEEN AND KIDNEY NECROSIS VIRUS (ISKNV) IN FARMED AND IMPORTED ORNAMENTAL FISH IN AUSTRALIA

N.J.G. MOODY*, P.G. MOHR, L.M. WILLIAMS, J. HOAD, D.M. CUMMINS, K.R. DAVIES, M.STJ. CRANE

CSIRO Australian Animal Health Laboratory, Geelong, Australia 3220

Viruses in the *Megalocytivirus* genus have not been detected in wild fish populations in Australia. However, viruses closely related to *Infectious spleen and kidney necrosis virus* (ISKNV) have been detected in imported ornamental fish. During a 2012 survey of a domestic ornamental fish farm in Queensland, scientists from the University of Sydney detected the presence of *Megalocytivirus* from healthy platys (*Xiphophorus maculatus*). Confirmatory testing of the samples at the AAHL Fish Diseases Laboratory confirmed the presence of an ISKNV-like virus. Subsequent screening of a further 265 healthy fish from the farm by real-time TaqMan PCR produced positive results in 14.7% of the samples with C_T values ranging from 27 to 44. Sequence analysis of conventional PCR amplicons targeting three different viral genome regions found that the viruses detected were indistinguishable from a reference ISKNV sequence. Only one nucleotide difference was identified over the >2680 nucleotides sequenced and analysed. Therefore, despite Australia's import requirements and quarantine measures, post-quarantine platys at an Australian ornamental fish farm were infected with a virus indistinguishable from ISKNV over the regions analysed.

An additional 272 tissues from randomly selected consignments of live imported ornamental fish were screened by real-time PCR in 2013 and 2014 as part of an Australian Government, Department of Agriculture trial to monitor the health of imported ornamental fish. The sampled consignments included over 20 species of ornamental fish from several genera. Real-time TaqMan PCR positive results were detected in 7% of the samples from five different genera; *Colisa*, *Poecilia*, *Pterophyllum*, *Trichogaster* and *Xiphophorus,* with C_T values ranging from 24 to 43. Confirmatory testing by conventional PCR and sequence analysis identified sequences that were either identical, or had one nucleotide different, to a reference ISKNV sequence.

The testing undertaken verified the usefulness of a real-time TaqMan PCR assay, originally designed to detect RSIV, for screening ornamental fish for the presence of ISKNV. A number of conventional PCR assays were also evaluated and compared for use as confirmatory assays to generate amplicons for sequence analysis.

THE VIRAL ENCEFALOPATHY AND RETINOPATHY VIRUS OF EUROPEAN SEA BASS *DICENTRARCHUS LABRAX:* NEW STRATEGIES FOR IMMUNIZATION

F. PASCOLI[1]*, A.TOFFAN[1], F. BUONOCORE[2], N. NUÑEZ[2], V. STOCCHI[2], S. PICCHIETTI[2], A. STRATMAN[3], G. SCAPIGLIATI[2]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, 35020 Legnaro (PD) Italy*
[2]*Università della Tuscia, 01100 Viterbo, Italy*
[3]*W42 Industrial Biotechnology GmbH, Germany*

Betanodaviruses are the causative agents of viral encephalopathy and retinopathy (VER), one the most important viral disease for global mariculture, especially for the European sea bass (*D. labrax*). Three vaccination trials were perfomed, one by intraperitoneally (IP) injection and two by bath, all followed by challenge with betanodavirus. In the IP trial, two groups of juvenile sea bass were IP immunized with inactivated virus solution. The results of the trial showed the detection of: i) serum antigen-specific IgM by ELISA and SN 30 days post infection (dpi); ii) *in vitro* VERv-induced gills leukocyte proliferation, and increase in proliferation of lectin-stimulated leukocytes; iii) a modulation in transcription levels of genes coding for IFN, Mx, ISG-2, and IgT in gills; iv) excellent protecion (RPS 81.5). In the bath trial, two groups of juvenile sea bass were immunized by immersion in inactivated virus solution for 2 minutes with and without a booster after 30 days. The results showed the detection of: i) no serum antigen-specific IgM 30 dpi; ii) neither *in vitro* VERv-induced gills leukocyte proliferation, nor increase in proliferation of lectin-stimulated leukocytes; iii) a modulation in transcription levels of genes coding for IFN, Mx, ISG-2, and IgT in gills; iv) immunohistochemistry (IHC) showed the presence of VERv in the gills of immunized fish; v) no protecion (RPS 2.4).
Finally, one group of juvenile sea bass, immunized by bath, was boosted by oral administration of feed added with killed *Pichia pastoris* yeast cells producing VER virus-like particles. Results showed: i) low, but detectable serum antigen-specific IgM 30 dpi; ii) *in vitro* addition of inactivated VERv induced proliferation of gills cells; iii) a significant modulation in transcription levels of genes coding for IFN, Mx, ISG-2, and IgT; iv) the presence of VERv antigens in the intestinal mucosa; v) a moderate protecion (RPS 29.6).
Overall, these results show the efficay of a single dose of inactivated virus vaccine against VER in sea bass administered by IP injection. On the other hand, solo bath immuniziation was unable to protect fish from mortality and clinical signs. Finally, preliminary positive results obtained with the oral boosting vaccination, although needing further study to be confirmed, suggest a new and potentially interesting approach to fight the disease.

The work was funded by the EU 7FP project TARGETFISH (Grant Agreement 311993).

DOES INFECTION OF SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) WITH PISCINE REOVIRUS (PRV) AFFECT THE OUTCOME OF SUBSEQUENT EXPOSURE TO INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV)?

S.C. JOHNSON[1]*, S. INKPEN[2], M.P. POLINSKI[1], J.C. BRADSHAW[1], M.L. RISE[2], K.A. GARVER[1]

[1]*Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo, Canada*
[2]*Department of Ocean Sciences, Memorial University, St. John's, Canada*

Concurrent infection (co-infection) of cells or organisms with two (or more) microorganisms is a common biological phenomenon. We are investigating the importance of viral co-infections as modulators of the immune response, including effects on innate and adaptive immunity, vaccine responses and resistance to disease in salmonids. As part of these studies, we examined the relationship between Piscine Reovirus (PRV) and Infectious Hematopoietic Necrosis Virus (IHNV) in Sockeye Salmon using laboratory challenges and transcript profiling by RNA Sequencing (RNA-seq) and RT-qPCR. Sockeye Salmon were challenged by ip injection of PRV positive tissue homogenate and subsequently bath challenged at 14 days post PRV exposure with IHNV. Unhandled fish and fish that were ip injected with PRV negative tissue homogenate served as controls. PRV challenge resulted in viral replication and the development of a sustained infection, without evidence of disease. RNA-seq analysis of head kidney samples collected at 14 and 21 days post PRV challenge identified few significant differences in the transcriptional response between PRV-challenged and control fish. Differentially expressed genes were identified as being primarily involved in a variety of pathways related to cell structure and function. The expression of genes involved in antiviral response (Mx, DHX58, IRF7 and ISG15) was also examined by RT-qPCR across a larger number of time points. With exception of small but significant increases in Mx, IRF7 and ISG15 in the PRV challenged fish at 2 days post challenge, there was no evidence of an antiviral response to PRV. Co-infection with PRV also had no significant effect on morbidity levels of Sockeye Salmon following challenge with IHNV when compared to controls. The transcriptional response of head kidney tissue to IHNV infection (7 days post IHNV challenge) was compared between PRV infected and negative controls using RNA-seq. Both PRV infected and non-infected groups showed well developed antiviral and inflammatory responses, the magnitude of which was effected by the IHNV titre. Our data suggests that infection with PRV does not affect how Sockeye Salmon respond to subsequent infection with IHNV. This is likely due in part to Sockeye Salmon having a very limited antiviral response to PRV.

CHARACTERISING IMMUNE RESPONSES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FOLLOWING THE ADMINISTRATION OF CELL-MEDIATED AND HUMORAL W/O ADJUVANTED VACCINES

K. VEENSTRA[1]*, S. RUSSELL[2], J.B. AROUS[3], C. SECOMBES[1]

[1]*University of Aberdeen, Aberdeen, UK*
[2]*Elanco Canada Ltd., Victoria, Canada*
[3]*SEPPIC, Paris, France*

Adjuvants are the helper substances that increase vaccine efficacy by enhancing the potency and longevity of specific immune responses to antigens. Most existing fish vaccines are presented in the form of oil-based emulsions delivered by intra-peritoneal injection; however, they are frequently associated with aggressive inflammatory responses resulting in clinically significant side-effects such as melanisation and adhesions. An *in vivo* trial was undertaken to develop a deeper understanding of vaccine formulations for fish, by trying to identify and characterize immune responses and side effects resulting from the administration of two adjuvants thought to induce humoral (Montanide™ ISA 763A VG) and cell mediated (Montanide™ ISA 761 VG) immune responses. Post-vaccination side effect scoring was followed by the removal of gill, spleen, head kidney and adipose tissue for RTqPCR gene expression analysis, and blood for antibody titres. 50 immune genes were examined with a focus on a) pro-inflammatory and b) adaptive immune related markers linked with possible Th_1, Th_2, Th_{17} and T_{reg} pathways. Unique immune gene profiles were observed among all four tissues as well as between humoral and cell mediated adjuvants. A strong up-regulation of pro-inflammatory genes which persisted over time, particularly in adipose, was observed. Side effects were more severe but antibody titres were significantly higher with the cell mediated vaccine. These findings provide valuable insights into inflammatory responses in fish following vaccination and will hopefully allow us to develop protocols enabling the rapid screening of novel vaccine adjuvant formulations in the future.

IMMUNOLOGICAL PARAMETERS CORRELATING WITH VIRAEMIC PERIOD OF SALMONID ALPHAVIRUS INFECTION IN ATLANTIC SALMON POST-SMOLTS

L. MOORE[1], J. JARUNGSRIAPISIT[1], G.L. TARANGER[1], T. KORYTÁŘ[2], B. KÖLLNER[2], T. O. NILSEN[3], S.O. STEFANSSON[4], H.C. MORTON[1], S. PATEL[1]*

[1]*Institute of Marine Research, Bergen, Norway*
[2]*Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany*
[3]*Uni Research AS, Bergen, Norway;* [4] *University of Bergen, Bergen, Norway*

Pancreas disease (PD) caused by salmon pancreas disease virus (SPDV), also known as salmonid alphavirus (SAV), is a serious problem in the salmon farming industry. Knowledge about how the physiological and immunological status of the salmon affects susceptibility to SAV infection is scarce, especially at the post-smolt stages when clinical cases of PD are seen. Our aim is to study the mechanisms of SAV infection and immune response to SAV at the cellular and molecular level by establishing a bath challenge model at the post-smolt stage. Salmon were infected by injection (IM) or bath immersion (BI). There was a significant difference in the susceptibility of post-smolts recently transferred to sea water compared to fish infected later. SAV infection status and immune gene expression in the recently smoltified experimental groups correlated with the viraemic period. Innate genes such as *IFNα*and *Mx* peaked at 7 or 14 dpi, whereas inflammatory markers showed a maximum expression at 14 or 21 dpi. In addition, increased expression of *TLR7*, *TLR8*, *MyD88*, and *IRF7* was observed. There were a few subtle differences in the temporal expression of these genes between the IM and BI models, which may be significant for the immune response in the field. This is perhaps due to the more natural viral infection route in the BI-model which might better represent a disease outbreak under field conditions. Immunohistochemistry with antibodies against MHC II, CD8 and IgM was carried out for semi-quantitative analysis of the cells involved during SAV infection. Flow cytometry analysis suggested that the blood of IM and BI infected fish contained an increased number of thrombocytes at both day 3 and 7 dpi.

TOLERANCE AND TRANSITION IN TROPHOZOITES: UNUSUAL AND ELABORATE BEHAVIORAL ADAPTIONS IN *SPIRONUCLEUS* SPP.

S.L. POYNTON[1, 2]*, K.W. WITWER[1], L. OSTRENGA[1], M.R. SAGHARI-FARD[2]

[1]*Johns Hopkins University School of Medicine, Baltimore, USA*
[2]*Leibniz Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany*

The diseases caused by *Spironucleus* species, intestinal flagellates of salmonids, gadids and cichlids, and systemic invaders of salmonids, are well known, and ultrastructure of trophozoites and cysts is documented. Key elements of the direct life cycle are known: trophozoites are typically in the intestinal lumen, (though extra-intestinal infections are known), cysts transmit the infection through the water, and upon ingestion the cycle is completed; reproduction is by longitudinal binary fission of trophozoites, and division in the cyst. However, we know relatively little about the tolerances and transitions of the *Spironucleus* parasites at different phases of their life cycle. Little is known about trophozoite behavior: how do they react to their environment, and interact with each other; what roles do these behaviors play in the life cycle?
We addressed these questions by observing *Spironucleus* trophozoites in culture. *Spironucleus vortens* (ATCC 50386, from Angelfish *Pterophyllum scalare*), were maintained in modified LYI medium, at 23 ^{o}C. Trophozoites were observed in a hemocytometer chamber and on a slide, and video recordings were made. *Spironucleus salmonis* from rainbow trout, *Oncorhynchus mykiss*, were maintained in minimal essential medium supplemented with newborn calf serum, at 10 ^{o}C. Trophozoites were observed in flasks and on slides. In *S. vortens*: (i) trophozoite distribution eventually conformed to the shape of the liquid in the hemocytometer chamber, with a 1.5 – 2.0 mm boundary, (ii) spherical swarms of trophozoites, 200 μm in diameter, formed, persisted for several minutes, and then disassociated. In *S. salmonis*, trophozoites adhered to each other by their posterior flagella, forming clusters prior to encystment. We interpret, for *S. vortens:* (i) adjustment in distribution as a response to chemoreception, probably of oxygen concentration, and (ii) swarming as intercellular communication leading to coordinated movement (possibly indicative of quorum sensing). For *S. salmonis*, multifunctional flagella initiate cell adhesion, leading to clusters of cysts, enhancing buoyancy and ingestion of numerous cysts, (presumably exceeding the minimum infective dose). Swarming is rare in flagellates, though common in certain animal groups; multi-functional flagella are rarely documented. Our new data showing *Spironucleus* spp. as an exceptional protist, may offer new insights into management and treatment.

SAPROLEGNIA DICLINA IIIA AND *S. PARASITICA* EMPLOY DIFFERENT INFECTION STRATEGIES WHEN COLONISING EGGS OF ATLANTIC SALMON, *SALMO SALAR*, L.

M. SONGE[1, 4]*, A. WILLEMS[2], J. WIIK-NIELSEN[1], E. THOEN[1], Ø. EVENSEN[3], P. VAN WEST[2], I. SKAAR[1]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*University of Aberdeen, Aberdeen, Scotland*
[3]*Norwegian University of Life Sciences, Ås, Norway*
[4]*International Livestock Research Institute, Nairobi, Kenya*

In this study, we addressed the morphological changes of eyed eggs of Atlantic salmon, *Salmo salar L.* infected with *Saprolegnia* from a commercial hatchery and after experimental infection. Eyed eggs infected with *Saprolegnia* spp. from 10 Atlantic salmon females were obtained. Egg pathology was investigated by light and scanning electron microscopy. Eggs from six of ten females were infected with *S. parasitica*, and two females had infections with *S. diclina* clade IIIA; two *Saprolegnia* isolates remained unidentified. Light microscopy showed that *S. diclina* infection resulted in the chorion in some areas being completely destroyed, whereas eggs infected with *S. parasitica* had an apparently intact chorion with hyphae growing within or beneath the chorion. The same contrasting pathology was found in experimentally infected eggs. Scanning electron microscopy revealed that *S. parasitica* grew on the egg surface and hyphae were found penetrating the chorion of the egg, and re-emerging on the surface away from the infection site. The two *Saprolegnia* species employ different infection strategies when colonizing salmon eggs. *Saprolegnia diclina* infection results in chorion destruction, while *S. parasitica* penetrates intact chorion. We discuss the possibility that these infection mechanisms represent a necrotrophic (*S. diclina*) versus a facultative biotrophic strategy (*S. parasitica*).

EFFECTS OF *LOMA MORHUA* (MICROSPORIDIA) INFECTION ON THE CARDIORESPIRATORY AND SWIMMING PERFORMANCE OF ATLANTIC COD *GADUS MORHUA* (L)

M. POWELL[1], A.K. GAMPERL[2]

[1]*Norwegian Institute for Water Research, Thormøhlens gate 53D, Bergen 5006, Norway*
[2]*Memorial University of Newfoundland, St. John's Newfoundland, Canada*

The microspridian *Loma morhua* infects Atlantic cod and results in the formation of xenomas within the gill filaments and the heart, and this is often associated with tissue inflammation. Given the importance of these two organs to metabolic capacity and thermal tolerance, the cardiorespiratory performance of Atlantic cod (*Gadus morhua*) with a naturally acquired infection of *Loma* was measured during an acute temperature increase ($2^{o}C\ h^{-1}$) from acclimation temperature ($10^{o}C$) to the fish's critical thermal maximum (CT_{Max}; $21.0 \pm 0.3^{o}C$). In addition, oxygen consumption and swimming performance were measured at $10^{o}C$ during two successive critical swimming speed (U_{crit}) tests. Comparison of metabolic and cardiac parameters with two previous studies on uninfected cod (Gollock et al., 2006 and Petersen and Gamperl, 2010) revealed that fish in the present study had: 1) higher and lower values for resting heart rate (f_H) and stroke volume (S_V)(by ~ 40%), respectively, and diminished values for maximum S_V (by 40%), cardiac output (by 70%) and metabolic rate (by 30%) in the CT_{Max} test; and 2) a diminished maximum MO_2 in the U_{crit} tests (by ~30%). However, oxygen consumption (MO_2) was only negatively correlated with gill xenoma density at $18^{o}C$ (not at cooler temperatures) in the CT_{Max} study, CT_{Max} was only slightly lower than in Gollock et al. (2006; $22.2 \pm 0.2^{o}C$) and not correlated with gill or heart xenoma density, and U_{crit} was nearly identical in this study to that reported in Petersen et al. (2010)($1.7\ bl\ s^{-1}$). Our results suggest that while *Loma* infection has negative effects on cod cardiac function at warm temperatures and on metabolic capacity, this species can largely compensate for these cardiorespiratory limitations.

ACTIVITY OF DIGESTIVE HYDROLASES IN FISH INFECTED WITH CESTODES

M.M. SOLOVYEV[1], G.I. IZVEKOVA[2]

[1]*Institute of Animal Systematics and Ecology, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*
[2]*Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Yaroslavl oblast, Russia*

Infection by parasites, in particular cestodes, may serve as one of the factors that affect the activity of digestive enzymes. Cestodes actively interact with the digestive system and influence it due to the morphological similarity of the worms' tegument to intestinal enterocytes, as well as due to the higher (compared to other helminthes) biomass and body surface that participate in the digestive–transporting processes. Infection by parasites may limit the host's ability to feed, which results in changes in the catalytic efficiency of intestinal enzymes. Cestodes lack the cavitary digestion, which is the first stage of digestion present in vertebrates. Parasites of this group possess the mechanisms of membrane digestion and active transport and are able not only to use the alimentary substrates of the host and products of the first stage of digestion, but also the host's enzymes adsorbed on the surface of the worm's body. The following fish species were studied: bream *Abramis brama* (Linnaeus), pike *Esox lucius* Linnaeus, and burbot *Lota lota* (Linnaeus). These fish were either non-infected or infected with cestodes *Caryophyllaeus laticeps* (Pallas), *Triaenophorus nodulosus* (Pallas) and *Eubothrium rugosum* (Batch), respectively. The data on the influence of cestode infection on the activity of digestive enzymes in their host fish are presented. The existence of proteinase and glycosidase activity gradients along fish intestines has been confirmed. Proteinases of hosts as compared to glycosidases were shown to be more responsive to cestode infection. The infection reduced the proteolytic activity in bream and burbot and increased it in pike. Both absolute and relative activity levels of the investigated enzymes changed. The infection provoked a redistribution of the relative content of various proteinase subclasses. In the infected burbot, the activity of proteolytic enzymes decreased to a greater extent in the places where worms are attached, i.e., pyloric caeca. A decrease in the activity of digestive enzymes of the hosts was observed even at low intensity of invasion.

The study was supported by the Russian Foundation for Basic Research, projects no. 15-04-02474 A and 13-04-00270 A.

HOST CONDITION AND ACCUMULATION OF METALS BY ACANTHOCEPHALAN PARASITE *ECHINORHYNCHUS GADI* IN COD *GADUS MORHUA* FROM THE SOUTHERN BALTIC SEA

M. PODOLSKA*, L. POLAK-JUSZCZAK, K. NADOLNA-AŁTYN

National Marine Fisheries Research Institute, Gdynia, Poland

Acanthocephalan parasites are sensitive indicators of metal pollution, because of its remarkable capacity to accumulate heavy metals at concentrations magnitude higher than those found in its host tissues. Concentrations of the following metals: Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, P, Pb, Sr, Zn were evaluated in the liver and muscle tissue of cod *Gadus morhua* from the southern Baltic Sea as well as in the tissue of its acanthocephalan parasites *Echinorhynchus gadi*. The relation of metal concentrations in tissues of hosts and parasites was expressed in terms of a bioconcentration factor (BCF), as the ratio of the element concentration in the parasites to that in host muscle tissue or in the liver. Acanthocephalans accumulated mainly toxic metals (Cd, Pb) and also Sr, Ca, Na. Cadmium showed the *highest* bioconcentration in parasites *(BCF* >200) in comparison to fish muscle tissue. The relationship between concentration of toxic metals in the host-parasite system and Fulton's body condition factor (FCF) of cod was analysed. Significant, negative correlation was detected between FCF and the concentration of Cd ($p<0.001$) and Hg ($p=0.005$) in the liver of fish. In contrast, FCF was positively correlated with the concentration of Hg ($p=0.004$) in the tissue of *E. gadi*. Obtained results may suggests protective role of *E. gadi* against accumulation of some toxic metals in cod.

A NEW METHOD FOR ASSESSING POTENTIAL ANTI-SEA LICE MICROINGREDIENTS AND COPEPODID SEA LICE (*LEPEOPHTHEIRUS SALMONIS*) HOST LOCATION BEHAVIOUR

R.M. HEAVYSIDE[1, 2]*, T.B. ERIKSEN[1], T. CORNULIER[2], C.J. SECOMBES[2], C. MCGURK[1], A.S. BOWMAN [2]

[1]*Skretting Aquaculture Research Centre, Stavanger, Norway*
[2]*Integrative Environmental Physiology, IBES, University of Aberdeen, Aberdeen, UK*

Sea lice are a major problem to salmon aquaculture. Control is usually by means of pesticide treatment, but more sustainable and environmentally-friendly methods are actively being sought. To this end, we assessed a behaviour observation arena for screening potential novel anti- sea lice microingredients in salmon feeds.

Presented here is a novel method for assessing sea lice behaviour towards chemical cues allowing a greater range of behaviours to be assessed in numerous lice simultaneously. In any one trial, many (e.g. 100) copepodid stage sea lice (*Lepeophtheirus salmonis*) were presented with two potential cues in a flow-through system with the additional option of retreating into a neutral zone. After 5 minutes, lice within each zone were recorded as well as their degree of stimulation. Copepodid sea lice demonstrated that salmon-conditioned water, or components therein, were not as kinetically attractive as previously thought/indicated, but rather indicated arrestant behaviour.

Low salinity water, including freshwater, was highly attractive.

Two potential microingredients were tested in their pure form and also as an assumed component of salmon-conditioned water from Atlantic salmon that had been fed the ingredient. One such component significantly reduced lice attraction.

This work has validated a new method for relatively high-throughput assessment of lice responses to chemical cues. It is anticipated that this method will be adopted in the initial screening of potential anti-lice substances in the development of new health feed diets for the salmon farming industry.

POSTERS

P-001

GENETIC ANALYSIS OF THE COMPLETE G GENE OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) ISOLATES IN TURKEY

H. ALBAYRAK[1]*, H. ISIDAN[2], G. KALAYCI[3], E. OZAN[4], V. VAKHARIA[5]

[1]*Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
[2]*Faculty of Veterinary Medicine, Cumhuriyet University, Sivas, Turkey*
[3]*Bornova Veterinary Control Institute, İzmir, Turkey*
[4]*Veterinary Control Institute, Samsun, Turkey*
[5]*IMET, University of Maryland, Baltimore, MD, USA*

The Turkey salmonid industry was developed by importing breeding materials, a practice still in effect due to deficits in the national supply of roe. Importation of breeding materials is often associated with the transmission of pathogens. Viral hemorrhagic septicemia virus (VHSV) is an enveloped non-segmented, single-stranded, negative-sense RNA virus that belongs to the *Novirhabdovirus* genus of the family *Rhabdoviridae* that causes one of the most important finfish diseases, affecting over 70 marine and freshwater species. The virus infects wild fish and cultured salmonids, causing high mortality in trout and salmon. This virus causes economically significant diseases of farmed rainbow trout, in Turkey, which is often associated with the transmission of pathogens from European resources. In this study, moribund rainbow trout (*Oncorhynchus mykiss)* samples were collected during an outbreak of VHSV in a fish farm in Bolu province of Turkey in 2009. And also two VHSV strains were isolated from wild turbot (*Scophthalmus maximus)* in Trabzon province of the Black Sea region of Turkey during field survey; and we have sequenced the full length G genomes of three VHSV isolates, compared them to 25 previously published genomic seguences. Based on a full-length glycoprotein gene nucleotid sequence, Turkish VHSV isolates were classified into the class Ie of genotype I and most closely related to GE-1.2 isolate (97.1-97.5% nucleotid identity and 98.2-98.4% amino acid identity) which was found more than 30 years ago in Georgia. They could be an indigenous types of VHSV distributing in Black Sea. On the other hand, our isolates have 97.5-97.6% nucleotid identity and 98.8-99% amino acid identity with Finland, Denmark and Norway isolates which are classified under Ib and Id. These isolates may have originated from Europe. These results suggest that the Turkish VHSV isolates orginated from Europe and/or indigenous strains are circulated together and threaten to aquaculture industry.

MOLECULAR CHARACTERIZATıON OF THE ALMUS INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) ISOLATE FROM TURKEY

H. ALBAYRAK[1]*, E. OZAN[2], V. VAKHARIA[3]

[1]*Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
[2]*Veterinary Control Institute, Samsun, Turkey*
[3]*IMET, University of Maryland, Baltimore, MD, USA*

The Turkey salmonid industry was developed by importing breeding materials, a practice still in effect due to deficits in the national supply of roe. Importation of breeding materials is often associated with the transmission of pathogens. Infectious pancreatic necrosis virus (IPNV) is a bisegmented double-stranded RNA virus belonging to the family *Birnaviridae*, genus *Aquabirnavirus*, which is a major viral pathogen of salmonid fish. The virus infects wild fish and cultured salmonids, causing high mortality in juvenile trout and salmon. This virus causes economically significant diseases of farmed rainbow trout, in Turkey, which is often associated with the transmission of pathogens from European resources. In this study, market size rainbow trout (*Oncorhynchus mykiss)* samples (200g.) were collected during an outbreak of IPNV in a fish farm in Almus Dam Lake in Tokat province of Turkey in 2013. When mortality rate was appreciated the under the light of the clinical observations and of the RT-PCR results, mortality rate reached up to 50.0% in Almus Dam Lake and higher than previously reported outbreaks in Turkey. We have sequenced the full length VP2 genome of Almus IPNV isolate (KM972672), compared them to 20 previously published genomic seguences. Based on a full length VP2 gene nucleotid and amino acid sequences, Almus IPNV isolate falls within genogroup 5, serotype A2 strain SP, having 100 % nucleotide and amino acide identities with the strain NVI-015 (AY379740) exhibited mortality of approximately 90% of susceptible Atlantic salmon fry and induced severe pathological lesions. Turkey has imported rainbow trout roe from Norway. Both isolates have same virulence motifs. These results suggest that the Almus isolate may have originated from Norway and IPNV, which is highly virulent for Atlantic salmon caused high mortality in rainbow trout.

MOLECULAR PHYLOGENY: A TOOL TO UNDERSTAND THE EPIDEMIOLOGY OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) IN FRANCE OVER THE PERIOD 1987-2014

L. BELLEC[1], R. THIÉRY[2], J. COZIEN[3], J. CABON[4], T. MORIN[4]*, L. LOUBOUTIN[4]

[1]*Université Pierre et Marie Curie, MNHN UMR 7208 BOREA, Paris, France*
[2]*French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Sophia Antipolis, France*
[3]*IFREMER, Laboratoire Santé Environnement et Microbiologie (PDG-RBE-SG2M-LSEM), Plouzané, France*
[4]*French Agency for Food, Environmental and Occupational Health & Safety (ANSES), European University of Brittany, Plouzané, France*

Infectious hematopoietic necrosis virus (IHNV) is an aquatic rhabdovirus that causes devastating epizootics in salmon and trout species in the Western North America, Europe, and Asia. Its typically bullet-shaped virion encapsidates a non-segmented, negative-sense, single-stranded RNA of ~11,000 nucleotides. The linear genome encodes six proteins in the 3′-5′ order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L). The six open reading frames (ORFs) are separated by short intergenic junctions and transcribed as monocistronic mRNAs. Phylogenetic analyses based on complete or partial G gene sequences of IHNV isolates from worldwide endemic areas have defined five major genogroups: U (upper), M (middle), L (lower), E (Europe) and J (Japanese Rainbow trout). In this study, we investigated the genetic diversity of 93 IHNV strains isolated in France over a period of 30 years using maximum-likelihood and Bayesian approaches. Some specific groups can be clearly identified and strongly supported. Viral sequences displayed a low genetic diversity with mean nucleotide identity ranging from 95.4 to 100%, with a similar trend for amino acids (93.1 to 100%). A ratio d_n/d_s (non-synonymous to synonymous mutations) of 0.28 was obtained as well as a significant negative value for the Tajima test (-1.95), indicating a purifying and negative selection on the viral population. Comparison of phylogenetic trees with the map of outbreaks allows suggesting possible epidemiological links. Thus, high similarities (> 99.2%) were observed between strains isolated from outbreaks in a same geographical region over a period of 10 years, clearly suggesting probable recontaminations through persisting local reservoirs. On the other hand, high level of amino acid identity between strains isolated in different geographical area on a short period of time incriminates rather a fish movement between regions. Such approaches will provide new insights in the future to better understand the circulation of IHNV strains among a territory.

CHARACTERIZATION OF EELPOUT RHABDOVIRUS (ERV) ON CELL CULTURE

E. BLOMKVIST[1]*, A. ALFJORDEN[1], M. HAKHVERDYAN[1], T.S. BOUTRUP[2], H. AHOLA[1], F. LJUNGHAGER[3], Å. HAGSTRÖM[1], N.J. OLESEN[2], M. JUREMALM[1], M. LEIJON[1], J.-F. VALARCHER[1], C. AXÉN[1]

[1]*National Veterinary Institute, Uppsala, Sweden*
[2]*Technical University of Denmark, Copenhagen, Denmark*
[3]*Swedish Agency for Marine and Water Management, Gothenburg, Sweden*

In 2014 mass mortalities occurred in eelpout (*Zoarces viviparous*) along the Swedish south-east coast. A new rhabdovirus, subsequently named eelpout rhabdovirus (ERV) was identified as the cause. Cell cultures for virus isolation were done for two mortality outbreaks. Kidney, spleen and heart from three eelpouts in Outbreak#1 were pooled into one sample; whereas kidney, spleen, heart and brain from four eelpouts in Outbreak#2 were pooled per fish. Organs were put in EMEM, homogenized and inoculated onto 24-well cell culture plates with BF-2 and FHM cells, with dilution in two steps. Three wells were used as negative control. Cultures were incubated 14-21 days at 15°C, with passage each 7 days. Additional culture was done on EPC (Outbreak#1-2) and RTG-2 cells (Outbreak#1) by the same procedure, but supernatant from 7-day-old BF-2 cell culture was used as inoculum. Plates were monitored daily from day 3 for cytopathogenic effects (CPE).
CPE appeared on BF-2 cells day 5 post inoculation (p.i.) for all samples, with full CPE day 7 p.i. For Outbreak#1, CPE on FHM cells only occurred after inoculation with BF-2 culture supernatant. For Outbreak#2, CPE on FHM cells appeared on day 7 p.i, in one of three wells (two samples) and in all three wells for one samplem with full CPE day 14 p.i. EPC and RTG-2 cells did not produce CPE for Outbreak#1. For Outbreak#2 there was full CPE on EPC cells day 7 p.i. The CPE was not similar to other viruses seen in our lab. The cell cultures looked full of debris, holes only appeared in BF-2 culture. For FHM and EPC, cells just suddenly detached when the amount of debris was massive. Titration identified a 2.7×10^5 $TCID_{50}$/ml for BF-2 cells and 1.9×10^4 $TCID_{50}$/ml for FHM cells. Titration was not done for EPC culture.
Thus, for ERV isolation, BF-2 is the best of the four routinely used cell cultures. Since at least two cell lines should be used, we recommend a combination with FHM culture if ERV is suspected. If CPE occurs on BF-2 but not FHM, new FHM inoculation should be done with BF-2 supernatant.

IMPACT OF VIRAL ENCEPHALOPATHY AND RETINOPATHY (VER) INTRODUCTION TO CROATIAN MARINE FARMS

D. BRNIĆ*, S. ZRNČIĆ, D. ORAIĆ, I. LOJKIĆ

Croatian Veterinary Institute Zagreb, Croatia

Since the first outbreak of viral encephalopathy and retinopathy on two Croatian marine farms in 1995, losses caused by this devastating disease have not been recorded. During the summer of 2014, outbreaks of the disease characterized by abnormal swimming behaviour in European sea bass (*Dicentrarchus labrax*) were notified from five different farms along the coast. In all cases infected fry was imported from the same hatchery and the outbreaks started a week or a month and half following introduction of fry to farm cages. The sea temperature during outbreaks was ranging from 21 to 24^{o}C. Affected fish from all farms were showing similar symptoms; dark pigmentation, emaciation, hemorrhagic lesions on the jaws, eye opacity, skin lesions and congestion and haemorrhages on the brain meninges. Mortality rate was very high. Samples of fish were submitted to the laboratory examination and virological examination of the brain homogenate on the SSN-1 cell culture resulted with CPE. Real time RT-qPCR confirmed infection with Noda virus in all cases. Two partial fragments within RNA 1 and RNA 2 of each isolate were amplified and sequenced and genetic characterization was performed. Impact of the infection was studied on the heavily infected farm with recorded advanced symptoms and high mortalities. Samples of all categories of the sea bass and sea bream (*Sparus aurata*) were collected three weeks after the first detection of the virus. Feral fish swimming around the cages on the infected farm including mullet (*Mugil cephalus*), salema (*Sarpa salpa*), bogue (*Boops boops*) and mussel (*Mytilus galloprovincialis*) from the cage installation were also collected. All samples were analyzed for the presence of beta noda virus by cell culture technique and RT-qPCR. It was demonstrated that virus has spread only to the older categories of sea bass where clinical symptoms were observed and low mortalities were recorded (up to 5%) compared to yearling category which suffered mortalities from 20 to 50 %.

NODAVIRUS INFECTION ALTERS THE KISSPEPTINS PATHWAY IN EUROPEAN SEA BASS BRAIN

Y. VALERO[1], A. CUESTA[2], M. ARIZCUN[1], M.A. ESTEBAN[2], J. MESEGUER[2], E. CHAVES-POZO[1]*

[1]*Instituto Español de Oceanografía, Puerto de Mazarrón, Spain*
[2]*University of Murcia, Murcia, Spain*

In vertebrates, the kisspeptins pathway in the brain is an essential upstream regulatory element of the hypothalamus - pituitary - gonad (HPG) axis, which regulates reproduction. In European sea bass it has been described two forms of kisspeptin genes, kisspeptin 1 (*kiss1*) and kisspeptin 2 (*kiss2*), and two forms of G-coupled protein receptor for kisspeptins, *gpr54-1b* and *gpr54-2b*. The kisspeptins pathway activation is involved in the release of GnRH in the brain, which subsequently, is involved in the release of the gonadotropin hormones (LH and FSH) in the pituitary, and in turn acts in the regulation of the steroidogenesis and gametogenesis in the gonad. One of the pathogens that greatly affects the brain is nodavirus (VNNV), a known vertically transmitted virus, which causes the viral encephalopathy and retinopathy (VER) disease. Previous studies showed that, in addition to infect the brain and cause a considerable fish death in European sea bass specimens, VNNV is able to colonize and replicate into the gonad, altering the sex steroid hormone levels and triggering a high immune response into the tissue. In this study, we have determined the alteration on the expression levels of *kiss1* and *2*, *gpr54-1b* and *-2b*, *gnrh1*, *2* and *3* genes and also the GnRH receptor (*gnrhr2a*) gene in the brain of European seabass males upon VNNV intramuscular or intravitreally infection. This study represents an advance on our knowledge about the interaction between host and VNNV, needed to understand how this virus is able to avoid the immune response of adult fish to spread to the progeny.

Financial support by grants AGL2010-20801-C02-01, AGL2010-20801-C02-02, AGL2013-43588-P, RYC-2009-05441 (MINECO and FEDER), 04538/GERM/06 (Fundación Séneca, Región de Murcia) and fellowship to Y. Valero (Instituto Español de Oceanografía) is gratefully acknowledged.

IMMUNE RESPONSE AGAINST INTRAVITREALLY INJECTED NODAVIRUS IN EUROPEAN SEA BASS SPECIMENS

Y. VALERO[1], A. GARCÍA-ALCAZAR[1], M.A. ESTEBAN[2], B. BOUGHLALA[3], J. DE JUAN[3], J. MESEGUER[2], E. CHAVES-POZO[1], A. CUESTA[2]*

[1]*Instituto Español de Oceanografía, Puerto de Mazarrón, Spain*
[2]*University of Murcia, Spain*
[3]*Universidad de Alicante, Alicante, Spain*

Viral diseases are responsible for many economic losses in modern aquaculture producing high mortalities. The viral nervous necrosis virus (VNNV), a single stranded RNA virus, produces the viral encephalopathy and retinopathy (VER) disease that is considered one of the most serious viral diseases in marine aquaculture. The virus mainly affects the central nervous system provoking necrosis and vacuolation of the cells of the spinal cord, brain and retina. Although some studies localized VNNV in the retina cells, no studies deal with how the virus affects the vision function and whether the immune response is orchestrated in the retina. Thus, in European sea bass (*Dicentrarchus labrax*) retina, VNNV has been localized in the inner nuclear cell layer and ganglion layer adjacent to the circumferential germinal zone at the ciliary margin towards the iris. In infected European sea bass, the affection of the retina seems to be often very advance, provoking early vacuolation, especially in the inner nuclear and ganglionic cell layers. In this study we intravitreally infected healthy specimens of European sea bass with VNNV and analysed the pattern of expression of relevant immune-related genes with the aim of characterizing the immune response in the tissue and also determined the functionality of the tissue by means of ultrastructure alterations. Light-adapted fishes were sacrificed at different times (1, 4, 24, 72 hours and seven days). Eyes were extracted, and its retinas were dissected and processed for transmission-electron-microscopy study. Thin sections, from each retina, were observed and photographed in an Electron Microscope. From the first day, after virus inoculation, structural changes were found in the retina. The more relevant changes observed were swollen cone pedicles (hydropic degeneration), on the third day and a diffuse vacuolation in the inner plexiform layer, concurrent with disrupted myelin sheaths, in the optic nerve fibres layer.

Financial support by grants AGL2010-20801-C02-01, AGL2010-20801-C02-02 and AGL2013-43588-P and RYC-2009-05441 (MINECO and FEDER), 04538/GERM/06 (Fundación Séneca, Spain), Vigrob-137 (University of Alicante) and fellowship to Y. Valero (Instituto Español de Oceanografía) is gratefully acknowledged.

MOLECULAR DETECTION OF A PUTATIVELY NOVEL CYPRINID HERPESVIRUS IN SICHEL (*PELECUS CULTRATUS*) DURING A MASS MORTALITY EVENT IN HUNGARY

A. DOSZPOLY[1]*, M. PAPP[2], P.P. DEÁKNÉ[2], R. GLÁVITS[2], K. URSU[2], Á. DÁN[2]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary*

The family *Alloherpesviridae* includes the herpesviruses detected or isolated from amphibian and fish species. The family contains four genera, one of them the genus *Cyprinivirus* comprises the herpesviruses of cyprinids and that of the European eel. The genus contains four species accepted by the ICTV: *Cyprinid herpesvirus 1*, *Cyprinid herpesvirus 2*, *Cyprinid herpesvirus 3* and *Anguillid herpesvirus 1*.
In the summer of 2014, mass mortality of Sichel (*Pelecus cultratus*) was observed in Lake Balaton, Hungary. Cadavers of six Sichel were sent to our laboratory for histopathological and molecular investigations. Histological examination revealed degenerative changes within the tubular epithelium, mainly in the distal tubules and collecting ducts in the kidneys and multifocal vacuolisation in the brain stem and cerebellum. The routine molecular investigations showed the presence of the DNA of an unknown alloherpesvirus in some specimens. Subsequently, three genes were amplified and sequenced partially from the putative herpesviral genome (DNA polymerase, terminase, and helicase). Phylogenetic tree reconstruction, based on the concatenated sequence of these three genes, implied that the virus undoubtedly belongs to the genus *Cyprinivirus*. The sequences of the Sichel herpesvirus differ markedly from those of the three known cypriniviruses; putatively representing the fourth cyprinid herpesvirus species. As for the Sichel loss in Lake Balaton, toxicological examinations were not carried out, the bacterial investigations proved to be negative, the minor infestation of different parasites do not explain the massive mortality event. The presence of the novel herpesviral DNA was detected only in one-third of the examined specimens. The histopathological abnormalities found in the samples could be a result of simple degradation. Taken together these facts, a direct connection between the presence of the herpesviral DNA and the mass mortality of Sichel could not be revealed. The causative agent of the outbreak remains unknown, further virological and toxicological studies would be needed for answering this question. This work was supported by a grant provided by the Hungarian Scientific Research Fund (OTKA PD104315), and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

WHOLE GENOME SEQUENCING OF TWO HUNGARIAN RANAVIRUS STRAINS ISOLATED FROM BROWN BULLHEADS (*AMEIURUS NEBULOSUS*)

S.L. FARKAS[1], A. DOSZPOLY[1]*, R. BORZAK[1], K. BANYAI[1], T. JUHASZ[2]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*National Food Chain Safety Office, Veterinary Diagnostic Directorate, Budapest, Hungary*

Members of the genus *Ranavirus* in the family *Iridoviridae* infect lower vertebrates: fish, amphibians and reptiles. Ranaviruses are known to cause acute, systemic disease and are often associated with mass mortality events. In brown bullheads (*Ameiurus nebulosus*) the disease is generally severe, pathologic/histopathologic changes are dominated by necrotic lesions in the kidneys and the spleen, and haemorrhages in the gills, the skin and internal organs.
In Hungary in the last decade ranavirus infection was frequently detected by virus isolation and virus specific PCR assays in connection with haemorrhagic syndrome and high mortality of brown bullheads. In order to obtain more information about the strains circulating in Hungary we decided to determine the whole genome sequence of isolates 13051/2012 and 14612/2012. Viruses were propagated in EPC and BF-2 cell lines. After freezing and thawing the infected cell culture PEG 6000 solution was used to concentrate viral particles, then DNA was purified and the samples were prepared for next generation sequencing. Sequencing was carried out on a 316 chip using Ion Torrent semiconductor sequencing equipment (Ion Personal Genome Machine_ [PGMTM], Life Technologies). The genome sequences were assembled applying the CLC Bio software (http://www.clcbio.com).
Nucleotide sequence analysis revealed 99.7 and 99.9 % identity to the European catfish virus (ECV; JQ724856) available in GenBank. We could identify all described ORFs of ECV in both studied genomes. The highest variability was observed in ORFs 105R and 105L giving the possibility for differentiation between strains within the same virus species, phylogenetic calculations and epidemiological follow-up.

This study was supported by KTIA-AIK-12-1-2013-0017 Grant, the OTKA PD104315 and the Momentum program of the Hungarian Academy of Sciences (HAS).

CHARACTERIZATION OF AN OUTBREAK OF CARDIOMYOPATHY SYNDROME (CMS) IN YOUNG ATLANTIC SALMON (*SALMO SALAR* L.)

C. FRITSVOLD*[1], T.T. POPPE[1, 2], E. HOEL[3], T. TAKSDAL[1], A.B. MIKALSEN[2], H. SINDRE[1]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*Norwegian School of Veterinary Science, NMBU, Oslo, Norway*
[3]*Marine Harvest Norway ASA, Bergen, Norway*

CMS is a cardiac disease in farmed Atlantic salmon (*Salmo salar* L.), causing significant losses to the fish farming industry in Norway. It is typically diagnosed in fish about 3-4 kg, around 1.5 years post sea water transfer, hence quite close to harvest.
CMS has been reported in Norwegian aquaculture since 1985. In 2014, 107 outbreaks were reported by the Norwegian Veterinary Institute. This is the highest yearly number of CMS diagnosis ever reported. In 2009 it was shown for the first time that the disease was infectious. The causative agent, *piscine myocarditis virus* (PMCV), was identified in 2010. CMS is characterized by severe macroscopic and microscopic circulatory disturbances, caused by heart failure, or sometimes even cardiac tamponade, resulting from severe myocarditis.
In the early spring of 2014, CMS was diagnosed in dead Atlantic salmon of 1 kg, only 6 months after sea water transfer. No moribund fish were observed, and the CMS diagnosed fish were normal individuals of good condition. There was a minor increase in mortality rate on the site, which continued until slaughter.
A real-time RT-PCR for the detection of PMCV was performed on samples from the heart and revealed remarkably large amounts of virus in this organ with Ct-values as low as Ct 7. Based on the fact that the disease was diagnosed on much younger fish than usual with uncommonly high amounts of virus, the site was monitored with 6 additional samplings until slaughter. Histopathology was performed on heart and a selection of other organs from all samplings, and virus load in the heart and other organs was studied in detail. In addition, the PMCV variant on the site was sequenced and compared to virus from other CMS outbreaks collected from GenBank. The results of these investigations will be presented and discussed.

SEQUENCIAL HISTOPATHOLOGICAL STUDY OF AQUAREOVIRAL HEPATITIS IN ATLANTIC HALIBUT, *HIPPOGLOSSUS HIPPOGLOSSUS*

J. GU[1]*, R. JOHANSEN[2], H.R. SKJELSTAD[1]

[1]*Norwegian Veterinary Institute, Trondheim, Norway*
[2]*Pharmaq Analytiq, Norway*

Aquareoviral hepatitis associated with high mortality in farmed Atlantic halibut (*Hippoglossus hippoglossus*) juvenile has been reported in Norway, Canada and Scotland (Blindheim, Cusack, Ferguson). However, there were some differences in histopathological changes between these reports. The main difference was degree of inflammatory response and this could be related to different developmental stages of the disease in the different studies. In the present study we therefore present a sequential histopathological study of an aquareoviral hepatitis outbreak in a Norwegian halibut farm.

Histological samples of 5-15 moribund Atlantic halibut juvenile were sequentially collected in February (week 0), short time after start-feeding, March (week 3), April (week 7) and May (week 11). Characteristic syncytia formation in liver was consistently observed from week 3 to week 11. Liver samples collected at week 11 were examined by transmission electron microscopy (TEM) with detection of large amount of aquareovirus-like particle.

Based on the features of sequential lesions in liver, developmental stages of aquareoviral hepatitis were categorized as pre-acute, acute, sub-chronic and chronic. In the pre-acute phase in February, no apparent lesions was noted in any of organs apart from minimal food content in digestive tract, which was in line with clinical sign of anorexia. At week 3, 40 % of fish exhibited acute changes consisting of extensive syncytial giant cell formations and multifocal caseous necrosis in liver. There was no evidence of inflammatory response. At week 7, all fish showed disease in sub-chronic phase characterized by varying degree of infiltration of lymphocytes in liver, while lesions of syncytial giant cell formations and caseous necrosis still dominated. Simultaneously, small clusters of cells with eosinophilic plasma were firstly observed at this stage. At week 11, 4 of 5 fish developed disease into chronic phase characterized by severe granulomatous inflammation and more frequent presence of clusters of eosinophilic cells. The severity of syncytia formation and necrosis was relatively mild. Meanwhile, signs of hepatocyte regeneration indicated by presence of basophilic hepatic cord and mitosis figures became obvious. In addition, similar sequential lesions were parallel observed in pancreas, but the severity was generally mild compared to liver. According to our observations aquareoviral hepatitis reported in Canada and Norway and Scotland could be respectively categorized as acute and (sub-)chronic.

IDENTIFICATION OF MEMBRANE PROTEIN PORF44 OF ABALONE HERPESVIRUS

C.S. HUANG[1], M.S. CHEN[1, 3], S.T. KUO[2], P.H. CHANG[1]*

[1]*Institute of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan*
[2]*National Institute for Animal Health, Tansui, Taiwan*
[3]*Tzu Chi College of Technology, Hualien, Taiwan*

Abalone herpesvirus (AbHV) infection of cultured abalone *Haliotis diversicolor supertexta* has induced high mortality rates in Taiwan in 2003. The histopathologic study has revealed AbHV is prone to infect neurotic tissues and has caused ganglioneuritis in moribund abalone. The abalone herpesvirus ORF44 was amplified by PCR using template DNA extracted from the neurologic tissue of *Haliotis diversicolor supertexta* infected with AbHV. The full length of the gene is 300bp encoding an membrane protein which consists of 110 amino acids. The predicted molecular weight of AbHV 0RF44 is approxilately 12 kDa. A total of 12 potential O-glycosylation sites are found in this protein. Based on the analysis of the flexibility in second structure, hydrophilicity, surface probability and antigenic index of AbHV ORF44 protein, the B cell epitopes are predicted. The sequence encoding the major epitope domain and the complete coding sequence of AbHV ORF44 gene were subcloned into pET-21a (+) vector to construct recombinant plasmids, which then were transformed into E. coli Rosetta and expressed by IPTG inducement SDS-PAGE and Western blot results show that pORF44 can be highly expressed and the truncated AbHV ORF44 protein is mainly expressed as soluble and purified. A rabbit antiserum was prepared against a bacterial fusion protein that permitted detection of the predicted membrane protein encoded by ORF44 of AbHV. The abundant ORF44 gene product of abHV exhibited an apparent mass by using Western blot analyses. It could be localized in the infected cells by indirect immunohistochemistry.

STUDY OF IHNV, VHSV AND KHV INFECTIONS IN FERAL FISH IN THE SELECTED SLOVENIAN WATERS

V. JENČIČ[1]*, P. HOSTNIK[1], K. PLIBERŠEK[2], A. GRILC-FAJFAR[1]

[1]*University of Ljubljana, Veterinary Faculty*
[2]*Fisheries Research Institute of Slovenia*

In this research, the presence of three viruses, viral haemorrhagic septicemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV) and koi herpes virus (KHV) were analyzed. The presence of IHN and VHS viruses in salmonids from 31 rivers and streams from the entire area of Slovenia, were investigated. Sampling locations were selected according to the analysis of the epizootiological situation and their possible links to the fish farms in which the infection was diagnosed. Rainbow trout and brown trout were collected for the laboratory analyses. Samples were examined using virus isolation in cell cultures (EPC, RTG-2), molecular diagnostic techniques (RT-PCR, RT-qPCR) and serological method (virus neutralization test, VNT). The results of virus isolation in the cell cultures and the results of molecular diagnostic methods were negative. To detect antibodies to IHN and VHS viruses, sera of rainbow trout and brown trout were collected. Using VNT, antibodies to IHN virus were found in 3 out of 8 investigated waters. Antibodies against VHSV were not detected. For the presence of antibodies to KHV, carp were sampled in 21 selected ponds and lakes in Slovenia and proved in 28.5% of the investigated waters. The results of VHS in IHN virus infections in Slovene rivers and streams are relatively optimistic given that the investigated waters connected to the IHNV and VHSV positive fish farms. It is especially encouraging that neither VHS virus nor antibodies were found in Slovene waters, which give the basis for the eradication of this serious viral infection in the fish farms. However, the presence of KHV antibodies are higher than expected considering that with an exception of two sites no clinical outbreaks were reported before. Introduced virus neutralization test is reliable diagnostic tool for the assessment of health status especially of the feral fish population as there is no need to sacrifice the fish.

"KOI SLEEPY DISEASE" DUE TO AN INFECTION WITH CARP EDEMA VIRUS IN GERMANY

V. JUNG-SCHROERS[1]*, M. ADAMEK[1], F. TEITGE[1], J. HELLMANN[1], S.M. BERGMANN[2], H. SCHÜTZE[2], D.W. KLEINGELD[3], K. WAY[4], D. STONE[4], M. RUNGE[5], B. KELLER[5], S. HESAMI[6], T. WALTZEK[6], D. STEINHAGEN[1]

[1] *Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2]*Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute of Infectology, Greifswald, Germany*
[3]*Lower Saxony State Office for Consumer Protection and Food Safety, Veterinary Task-Force, Hannover, Germany*
[4]*Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, Dorset, UK*
[5]*Lower Saxony State Office for Consumer Protection and Food Safety, Food and Veterinary Institute Braunschweig / Hannover, Germany*
[6]*University of Florida, College of Veterinary Medicine, Department of Infectious Diseases and Pathology, Gainesville, Florida, USA*

Diseases associated with a viral infection can induce high morbidity and mortality in fish with global trade causing a great potential for their spread. Infections with carp edema virus (CEV), a pox virus, are known from Japanese koi populations since 1974. A characteristic clinical sign associated with this infection is lethargy and therefore the disease is called "koi sleepy disease". Diseased koi also show swollen gills, enophthalmus and skin lesions. The mortality can rate up to 80%. Disease outbreaks seemed to be restricted to Japan for a long period of time. However, during the last years clinical outbreaks of koi sleepy disease also occurred in European countries like the UK, the Netherlands and Austria.
In spring 2014 koi from different ponds in northern Germany showed lethargic behavior, skin ulcers, inflammation of the anus, enophthalmus and gill necrosis. In all cases, new koi had been purchased earlier that spring from the same retailer. Koi were examined for ectoparasites, bacterial and viral infections (cyprinid herpesviruses in general, Koi Herpesvirus and Carp Edema Virus). In most of the cases parasites were not detected and only opportunistic bacteria were isolated. No cytopathic effect was observed in cell cultures, and all samples gave negative results for cyprinid herpesviruses. By analyzing gill tissues for CEV by a PCR designed by Oyamatsu et al. (1997), PCR products of 547 bp could be amplified. By sequencing of the products an outbreak of Koi Sleepy Disease was confirmed. To optimize diagnostics, a qPCR protocol for detection of CEV was developed. The reliable detection limit of this qPCR is lower than 10 copies. Therefore the qPCR gives a significant advantage over the end point PCR having a noticeably higher sensitivity.
This is the first description of a clinical outbreak of "koi sleepy disease" due to an infection with Carp Edema Virus in Germany. To avoid transmission of CEV to common carp testing of CEV should become part of fish disease surveillance programs in European countries.

SPLEEN AND KIDNEY NECROSIS VIRUS IN ORNAMENTAL FISH IN GERMANY

V. JUNG-SCHROERS[1]*, M. ADAMEK[1], P. WOHLSEIN[2], J. WOLTER[3], H. WEDEKIND[4], D. STEINHAGEN[1]

[1]*Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2]*Institute for Pathology, University of Veterinary Medicine, Hannover, Germany*
[3]*Medical practice for ornamental fish, Berlin, Germany*
[4]*Institute for Fisheries, Bavarian State Research Centre for Agriculture, Starnberg, Germany*

Megalocytiviruses, a group in the family *Iridoviridae*, can be divided into three major subgroups: Infectious Spleen and Kidney Necrosis virus, Red Sea Breem Iridovirus and Turbot Reddish Body Iridovirus. They cause systemic infections in a wide range of marine and freshwater fishes with heavy losses. Affected are aquaculture and ornamental fishes.
In 2013 high losses in angelfish (*Pterophyllum scalare*) were seen in the facilities of a number of German retailers, which imported ornamental fish from Asia. The fish showed symptoms like anorexia, lethargy, gill swelling and skin alterations. Mortality rates up to 100% occurred. Antibiotic treatment was not helping and parasites could not be detected. Samples from one of those retailers were analyzed systematically. In total five groups of fish, two groups of angelfish and three groups of platys were examined. Samples were taken from one group of angelfish, which died in the retailers facilities to 100% in 2013 and from living angelfish and platys (*Xiphophorus maculatus*), which already showed similar symptoms in 2014.
In none of these fish parasites could be detected. In one group of platys mycobacteria could be found. In all other fish no bacteria or only opportunistic bacteria in low numbers could be detected. Histological examination resulted in profound alterations in almost all internal organs. Especially necrosis in spleen, kidney and liver and a high number of hypertrophic, intensively pink stained cells which were distributed in liver, spleen and kidney, could be detected in all samples from both angelfish and platys. Tissue samples were analyzed by electron microscopy and high numbers of virus particles could be seen. We performed PCRs for detection of iridoviruses, as infections were described in angelfish. A PCR designed by Kurita et al. (2012) gave products of 777 base pairs. By sequencing these products an outbreak of Spleen and Kidney Iridovirus was confirmed.
This is the first report of the detection of Spleen and Kidney Iridovirus in ornamental fish in Germany. Because of the severe progressive form of this infection and the high mortalities, it should be considered to keep ornamental fish separately in quarantine and to examine them directly after import.

MONITORING FOR THE MAIN FISH VIRUSES IN KAZAKHSTAN

A. KYDYRMANOV*, A. SEIDALINA, S. SULEIMENOVA, K. KARAMENDIN, YE. KASSYMBEKOV

Institute of Microbiology and Virology, Almaty, Kazakstan

Up to date in Kazakhstan, the research on infectious diseases of fish has not yet been conducted. Therefore, virological monitoring of fishing ponds and fish in Kazakhstan is an important task, which is of great economic importance.

More than 340 samples of internal organs, gills, gonad products and blood were collected for virological survey from fish of 12 species belonging to *Esocidae, Cyprinidae, Salmonidae, Mugulidae, Persidae* families in West, East, and South-East Kazakhstan during the spawning and foraging in 2014.

A PCR-screening for the fish viral pathogens in water bodies of Kazakhstan was held for the first time. OIE listed fish pathogens such as spring viraemia of carp (SVC), infectious haematopoietic necrosis (IHN), viral haemorrhagic septicaemia (IHS) were not found in Kazakhstan. Five samples of *Cyprinidae* fishes without signs of infection from the Lake Balkhash (South-East Kazakhstan) were positive for *koi* herpes virus. Materials collected in the East Kazakhstan region from Lenok *(Brachymystax lenok),* were examined for the presence of nucleic acids of VHS and IHN pathogens. As a result of PCR analysis, viral RNAs in these samples were not detected, despite the presence of morphologic manifestations of suspected infections.

Diagnostic research was carried out with samples taken from mullets (*Mugil cephalus)* during their mass death in the Kazakh sector of the Caspian Sea in August of 2014. It is considered that the increased water temperature affects the outbreaks so called "summer disease" (viral encephalopathy and retinopathy - VER), caused by *Betanodavirus* genus of the *Nodaviridae* family. Swimming behavior of affected fish, abnormally high water temperatures (up to +28°C) and the mortality of only one species of fish fauna of Caspian Sea indicated on possible outbreak of VER. The expected PCR products of 255 and 341 base pairs are characterizing VER in the studied samples were not identified.

Increasing importation of fish breeding stock regardless of the epizootic situation in the area of origin requires permanent monitoring of fish viral diseases in Kazakhstan.

ISOLATION OF VHS AND IHN FROM RECENT OUTBREAKS ON CROATIAN RAINBOW TROUT FARMS

D. ORAIĆ[1]*, S. ZRNČIĆ[1], D. BRNIĆ[1], N. VENDRAMIN[2], S.S. MIKKELSEN[2], M.S. BRUUN[2], A. TOFFAN[3], N.J. OLESEN[2]

[1]*Croatian Veterinary Institute, Zagreb, Croatia*
[2]*Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*

Croatia is implementing surveillance programme with regard to viral listed diseases and until 2013, only IPNV was present on several salmonid farms. In July 2013, virus causing viral hemorrhagic septicaemia was detected in rainbow trout (*Oncorhynchus mykiss*) fingerlings from single farm. During 2014, virus was spread to another two farms. Affected fish showed exophthalmia, pale gills, distended abdominal wall with haemorrhages on the skin and in the eyes. On autopsy, haemorrhages on the liver, pyloric caeca, swim bladder, intestine, in dorsal musculature and enlarged spleen were recorded. VHS virus was isolated on cell cultures and identified by ELISA. The diagnosis was confirmed by RT-PCR and partial sequencing of nucleocapsid and glycoprotein genes was performed. The obtained nucleotide sequences were typed as genotype Ia, subtype 2, grouped into subclade Pol II that includes isolates from Poland and Slovenia. In December 2013, virus of infectious haemotopoietic necrosis was detected in samples from newly established rainbow trout farm, submitted as a part of routine monitoring programme. Later, during the 2014, the disease has spread to another three farms. Samples of affected fish showed exophthalmia, pale gills, and sometimes haemorrhage on the skin. Necropsy revealed abundant haemorrhages on the fat tissue, pyloric caeca, peritoneum and intestines and in single specimen on the liver and in dorsal musculature. Examination of organ pools on the cell culture resulted by virus isolation and identification was performed by ELISA. Obtained results were confirmed by RT-PCR and extended mid-G region (615 instead of 303 nucleotids) was sequenced and according to given results we concluded that it is IHNV isolate belonging to M group. Epidemiological investigation included susceptible species from open waters supplying infected farms as well as rivers downstream. All samples from open waters were negative. Further research should be performed to investigate the source of infections.

EFFECT OF TEMPERATURE ON THE EXPRESSION OF IFN-1 (α), STAT-1 AND MX-1 GENES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EXPOSED AT INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV)

D. ARGUEDAS[1, 4], A. ROMERO[2, 3], R. ENRÍQUEZ[2], S. MARTÍNEZ[1], B. VALLADARES[1], C. ORTEGA[1]*

[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México; Toluca, México*
[2]*Facultad de Ciencias Veterinarias, Universidad Austral de Chile; Valdivia Chile*
[3]*Centro FONDAP, Interdisciplinary Center for Aquaculture Research (INCAR), Chile*
[4]*Laboratorio LARED, Universidad Técnica Nacional, Guanacaste, Costa Rica*

The infectious pancreatic necrosis virus (IPNV) is the causative agent of an acute illness well characterized in salmonids worldwide. Clinical signs and mortality rates are dependent on several factors such as the viral dose, the age of the fish, the water temperature, among others. An experimental study was conducted to measure the effect of temperature on the gene expression profile of IFN-1(α), STAT-1 and Mx-1 in rainbow trout fry, exposed to IPNV. Fry (n=198) were exposed at 8, 12 and 16°C, and samples were taken for 21 days to determine the virus titer and gene expression. In the first 11 days the greatest viral titer was recorded at 8°C compared with the values obtained at 12 and 16°C. At 8°C, there was a significant increase on day 4 of mRNA Mx-1 (t-test, $p<0.05$), time in which the viral titer began to decrease. Furthermore, as the viral titer increased, STAT-1 and Mx-1 ($r=0.91$) and ($r=0.96$) increased, respectively. The animals were able to recover from day 4 from some of the symptoms of IPN. Clinical disease was developed only in fish exposed to 12°C and all died between days 6 and 14, despite the highly significant increase shown in the average expression level of Mx-1, compared with the values recorded at 8°C and 16°C (Tukey, $p<0.0001$). Additionally, the expression profiles of IFN-1(α) and STAT-1 decreased completely (~0.016) and (~0.020 times) on day 7. The highest expression level of IFN-1(α), occurred at 16°C (Tukey, $p<0.0005$). Fry exposed at 16°C were normal during the experiment. IFN-1(α) possibly generated a protector effect from day 2 when they showed a significant expression increase compared with the results at 8°C and 12°C (t-student, $p<0.0001$); however, STAT-1 was not significantly affected by temperature, although the highest average expression value was recorded at 16°C. Our research supports the expression of relevant anti-viral response genes as IFN-1(α), STAT-1 and Mx-1 are physiologically modulated by the water temperature, directly influencing the development of the IPN disease in rainbow trout.

RECURRENT VNN OUTBREAKS AFFECTING WILD GROUPERS IN THE MEDITERRANEAN BASIN

V. PANZARIN[1]*, M. ABBADI[1], M. BAUD[2], L. BIGARRÉ[2], H. KARA[3], G. FUSCO[4], G. PURPARI[5], G. CATTOLI[1], A. TOFFAN[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy*
[2]*Agence National de Sécurité Sanitaire, Plouzané, France*
[3]*University Badji Mokthar, Annaba, Algeria*
[4]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli, Italy*
[5]*Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy*

Viral nervous necrosis (VNN) is a serious infectious disease caused by betanodaviruses. Their genome is constituted by two ssRNA molecules. The RNA1 gene encodes the viral replicase and gives rise to the sub-genomic transcript RNA3 translated into protein B2. The RNA2 segment encodes the capsid protein. Based on the phylogenetic analysis of the RNA2 molecule, betanodaviruses have been classified into four species: RGNNV, SJNNV, BFNNV, TPNNV. Due to the segmented nature of their genome, fish nodaviruses can undergo reassortment events resulting in antigenic shift. In the Mediterranean basin, the occurrence of RGNNV, SJNNV and reassortants RGNNV/SJNNV and SJNNV/RGNNV have been documented. Betanodaviruses represent a major problem for marine aquaculture, but they are also considered as an ecological hazard for wild fish. Indeed, in the past years recurrent and severe VNN outbreaks have been reported in Mediterranean wild groupers (*Epinephelus spp.*).

In our survey, we collected genetic and epidemiological data related to documented VNN episodes affecting wild groupers from Algeria, Greece, Italy and Tunisia. Overall, 42 RNA1 and RNA2 partial sequences were obtained. The phylogenetic analyses indicated that all the strains detected in wild groupers were typed as RGNNV. The RNA1 topology showed that groupers betanodaviruses were distributed within six different genetic clusters, corresponding to specific geographic origins. On the contrary, the RNA2 phylogenetic tree highlighted that betanodaviruses from different locations were grouped within the same cluster, thus suggesting a low antigenic diversity among viral strains circulating in wild Mediterranean groupers. P-distance estimated among wild groupers betanodavirus sequences highlighted a higher genetic variability of the polymerase gene in comparison to the coat protein gene (0-7.7% diversity for RNA1 vs 0-4.5% diversity for RNA2). In few cases, the high similarities between sequences from wild groupers and farmed seabass reared in close proximity of the observed outbreaks suggested inter-species viral exchange, although it was not clear which of the species was a source of infection for the others.

The collection of genetic and epidemiological data, and the report of new disease outbreaks are crucial to deeply understand betanodavirus ecology in wild groupers and to develop strategies for preserving the Mediterranea endangered wild fauna.

EXPERIMENTAL INFECTION OF STONE LOACH (*BARBATULA BARBATULA*) AND HYBRID OF STARLET (*ACIPENSER RUTHENUS*) AND BELUGA (*HUSO HUSO*) WITH CYHV-3

V. PIAČKOVÁ[1]*, A. POSPÍCHAL[1], D. POKOROVÁ[2], S. RESCHOVÁ[2], T. VESELÝ[2]

[1]*University of South Bohemia Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic*
[2]*Veterinary Research Institute, Brno, Czech Republic*

Cyprinid herpesvirus 3 (CyHV-3) is a causative agent of highly contagious disease – koi herpesvirus disease (KHVD) and can cause significant losses in fish stock of susceptible species. Virus is restricted to koi carp and common carp, but last investigations showed that other cyprinid or even non-cyprinid species can be asymptomatically susceptible to this virus and can play a role of a potential carrier of CyHV-3 or they can contribute to biological conservation of this virus in environment.

In this study, stone loach (*Barbatula barbatula*) and sterbel (sterlet *(Acipenser ruthenus)* × beluga *(Huso huso)*) were chosen for testing of susceptibility to CyHV-3. These two species were cohabited with infected koi carps in aquaria (primary challenge test). Naïve koi carps were used as a positive control of infection. In 15 dpi, some of stone loach and sterbel were transferred to another naïve koi carps (secondary challenge test). All fish (dead during the test as well as surviving fish which were killed at the end of the test) were sampled and checked by nested PCR.

During the primary challenge test, 100% of koi carp died and 95% of them were found CyHV-DNA positive by PCR. One stone loach (5%) and one sterbel (5%) also died (in 10 and 14 dpi respectively) but they were CyHV-3 DNA negative. At the end of primary challenge test (in 30 dpi), surviving fish were sampled and checked by PCR. 35% of stone loach and 10% of sterbel were found CyHV-3 DNA positive.

During the secondary challenge test, 100% of transferred stone loach as well as cohabited koi carp died in two weeks in first group, 60% of koi carp and none sterbel died in second group. Mortality in secondary challenge test had to have other aetiology because all fish were CyHV-3 DNA negative. It could mean that 15 days was not sufficient time for virus to enter cohabited "non susceptible" species but it is necessary to check it.

This study was funded by Ministry of Agriculture of the Czech Republic (Grant No MZE-NAZV QJ1210237), by Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA" (No. CZ.1.05/2.1.00/01.0024) and "CENAKVA II" (No. LO1205 under the NPU I program)" and the Grant Agency of USB GAJU 087/2013/Z.

ANTIVIRAL ACTIVIITY OF MICROALGAL EXTRACTS AGAINST KOI HERPESVIRUS (KHV)

M. REICHERT[1]*, C. LINDENBERGER[2], R. BUCHHOLZ[1]

[1]*Friedrich-Alexander University of Erlangen-Nürnberg, Erlangen, Germany*
[2]*Friedrich-Alexander University of Erlangen-Nürnberg, Campus Busan, South Korea*

Although koi herpes virus (KHV) has a history of causing carp farmers sever economic losses there are still no drugs against it available on the market. Thus, the aim of this study was to find novel substances from microalgae showing antiviral activity against this virus by monitoring infection inhibition and cytotoxic effects in common carp brain (CCB) cells (Neukirch *et al.* 1999). In order to reach this aim, at first, native fresh water and marine eukaryotic microalgae from different locations in South Korea were isolated, identified, using morphological and genotyping methods, and screened for their antiviral properties. Among 80 tested isolates, the extracts of 7 algae showed antiviral activity against KHV (isolated in Poland, Dr. Magdalena Stachnik, 2013) at a multiplicity of infection (MOI) of 0.45 16-20 days *post infection* and no cytotoxicity towards CCB cells. In contrast, standard substances such as Ganciclovir, Cytosine ß-D-arabinofuranoside and Cyclohexamide, applied at 25 µg/mL, showed growth inhibitory or cytotoxic effects on CCB cells and no antiviral activity at the same MOI indicated by expression of all chosen KHV genes (ORF 3L/R, ORF 55, ORF 56, ORF 92 and ORF 114) 16 days *p.i.*. Furthermore, *Arthrospira platensis*, well known for the antiviral activity of intra- and extracellular compounds towards human cytomegalovirus (König, 2007) was investigated. Of all different extracts from this species the highest antiviral activity with no cytotoxic effects were observed for the dialyzed fraction of the exopolysaccharides (EPS). However, EPS have inhibitory impact on growth of the CCB cells with $IC_{50,\ growth}$ at 59 µg/mL. Additionally, it was found that EPS prevent the KHV replication in CCB cells at different MOI values (> 4.5) and at concentrations of 18-37 µg/mL up to 22 days *p.i.*. The virus inhibiting concentration ($IC_{50,\ inhibition}$) of EPS was determined to be > 5.5 µg/mL.
To sum it up, the presented study show very promising results in respect to finding novel compounds of microalgal origin that can prevent and/or treat fish infection by KHV. Nevertheless, further studies are needed in order to prove their effectivity for application in animals.

We kindly thank Dr. Magdalena Stachnik, Department of Fish Diseases, National Veterinary Research Institute in Pulawy (Poland), for providing the virus isolate KHV-P. This work was supported by KIAT, South Korea, which is greatly appreciated by the authors.

THE PRESENCE OF VIRAL PATHOGENS IN EUROPEAN EEL POPULATION FROM VISTULA LAGOON (BALTIC SEA)

M. STACHNIK[1]*, T. NERMER[2]

[1]*National Veterinary Research Institute, Puławy, Poland*
[2]*National Marine Fisheries Research Institute, Gdynia, Poland*

Vistula Lagoon is a area of mixing of fresh water and saline water from the Baltic Sea. Rapid urbanization and the development of industry and tourism without adequate waste-water management subjected this environment to new disturbances including eutrophication, massive algae blooming, and the decline of macrophytes. Decreases in catches of the most valuable fish species occurred simultaneously. Eel occupies a special position in Vistula Lagoon fisheries, but it is no longer a primary source of income for Polish fishers because of drastic decreases in biomass. Stocking cultured eel was begun again in 2005 in the Polish part of the lagoon, and since 2011 additional measures have been implemented as part of the European Eel Management Plan, establishing measures for the recovery of the stock of European eel. Diseases are an important cause of losses and decreased production rates in freshwater eel farming, and have been suggested to play a contributory role in the worldwide decline in wild freshwater eel stocks. One of the most commonly detected pathogenic viruses of European eel is anguillid herpesvirus 1 (AngHV1). In our study we present results of investigation for presence of pathogenic viruses in European eel sampled in September 2014 from Vistula Lagoon. Samples from 32 specimen (average length 62 cm and 565 g) were prepared from pooled spleen, kidney and gills of each fish. Otoliths have been used to determine the age of fish. Research material was inoculated on one-day old eel kidney 1 (EK-1) cell monolayers in 24-well plates and incubated in 20±2°C. CPE-inducing agent was identified as anguillid herpesvirus 1 by real-time PCR method in 5 animals. The age of pathogen carriers was described as 4,5 and 9 years, what suggests that stocking material introduced at that time to Vistula Lagoon could be infected with anguillid herpesvirus.

SCREENING FOR VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN ATLANTIC COD (*GADUS MORHUA)* FROM THE BALTIC SEA

M. STACHNIK[1]*, K. NADOLNA-AŁTYN[2]

[1]*National Veterinary Research Institute, Puławy, Poland*
[2]*National Marine Fisheries Research Institute, Gdynia, Poland*

Viral hemorrhagic septicemia (VHS) is a severe viral infection causing great losses in fish farming but infects also a wide range of marine species. Ulcer syndrome is one of the more common diseases affecting Baltic fish. VHSV has been associated with skin ulcers in both species - Pacific cod (*Gadus macrocephalus)* and Atlantic cod (*Gadus morhua*), but whether this represents an opportunistic infection or if VHSV plays a role in the aetiology of this condition remains unclear. This study presents results of screening for viral hemorrhagic septicemia pathogen in commercially very important fish species for the Baltic Sea - *Gadus morhua*. Specimen of Atlantic cod were sampled in three areas of Polish Exclusive Economic Zone of the Baltic Sea in February 2015. Fish were kept in temperature about 4°C until sampling of organs. Standard ichtyological analysis has been performed and biological parameters of each fish have been described. Samples consisted of kidney, spleen and liver were diluted in transport medium and frozen in -20 °C immediately after collection of organs for further analysis. After inoculation on bluegill fin (BF-2) cells, chinook salmon embryo (CHSE-214) and epithelioma papillosum cyprini (EPC) cells, a cytopathic effect induced by virus was expected. In total, samples of pooled internal organs from 93 individuals of *Gadus morhua* species (average length 40 cm and 580g of weight) were investigated by cell line isolation and RT-PCR. Presence of VHS virus was not confirmed in any of tested samples.

SUSCEPTIBILTY TO VHS AND IHN INFECTION OF COMMONLY FARMED FRESHWATER SALMONID SPECIES IN POLAND

M. STACHNIK[1]*, M. JESIOŁOWSKI[2]

[1]*National Veterinary Research Institute, Puławy, Poland*
[2]*Dąbie Fish Hatchery, Dąbie, Poland*

Salmonids are one of the most important fish groups in aquaculture. Fish farming is characterized by a high density and high efficiency, which leads to the threat of infectious diseases, particularly dangerous viral diseases. Rainbow trout is one of most common freshwater species of salmonids, characterized by rapid weight gain and increased resistance to poor environmental conditions in high density during breeding. Unfortunately, it is highly susceptible for viral infection, such which brings to high losses in aquaculture facilities. To improve trout aquaculture including growth performance or higher immunity, manipulation of genetic material like polyploidization or crossbreeding is performed. Hybrids may be favored especially for their resistance to certain viral diseases, e.g. between rainbow trout and arctic charr or brook trout are often described as less susceptible to viral infection comparing to RT. This study was performed in order to describe susceptibility of 4 commonly farmed salmonids species and their hybrids: rainbow trout (RT), brook trout (BT), hybrid of brook trout x arctic charr (BxA) and rainbow trout x brook trout (RxB) for VHS and IHN virus infection. Fish were exposed to pathogen by bath with VHSV and IHNV isolates from Polish outbreaks. We used to infect 60 individuals of species and hybrids in tanks and an untreated control. Daily observations were made to record the potential symptoms of disease or mortality. Weekly, samples of organs were collected from 10 fish from each tank to isolate the viruses (by method recommended by EURL and OIE). Approximately 2 weeks after disappearance last symptoms of infection to tanks with hybrids were added RT to determine their potential of carrying and transmitting the virus to trout. We observed very low mortality in RxB comparing to RT and no mortality in BxA at all. Altough, we isolated VHS virus from rainbow trout added to RxB and IHN virus from trout added to BxA. These results suggest that breeding of hybrids may be a good solution for farms earlier infected with virus or located in areas with high risk of possible infection. However, their potential as carriers of virus transmission it should be taken into consideration.

EXPERIMENTAL INFECTION OF FRESHWATER SALMONID SPECIES WITH HIRAME RHABDOVIRUS (HIRRV)

E. BORZYM[1], M. STACHNIK[1]*, J. MAJ-PALUCH[1], M. MATRAS[1], M. BAUD[2], L. BIGARRÉ[2]

[1]*Department of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland*
[2]*Unité Pathologies Virales des Poissons, ANSES, Université Européenne de Bretagne, technopole Brest-Iroise, Plouzané, France*

Hirame rhabdovirus (HIRRV) is one of the four recognized species within the *Novirhabdovirus* genus. HIRRV was isolated during an outbreak on cultured flounder and ayu in Japan and stone flounder in China. Furthermore, it was shown to be pathogenic on a range of salmonids species, including rainbow trout, experimentally challenged in freshwater. The major clinical signs of HIRRV infection were congestion of the gonads, focal hemorrhage of the skeletal muscle and fins and accumulation of ascitic fluid. The HIRRV was isolated form farmed grayling and brown trout in Poland, during the outbreak with clinical sings in this farm. Initial experiments involved the experimentally infection of rainbow trout and grayling with this virus. During which demonstrated the sensitivity of these fish to the virus, with clinical symptoms and mortality. Second experimentally infection of HIRRV concerned 4 species and hybrids of salmonids: rainbow trout (RT), brook trout (BT), hybrid of brook trout x arctic char (BxA), hybrid rainbow of trout x brook trout (RxB). 50 fish of each taxon after acclimatization, were infected by immersion in bath with HIRRV. An untreated control was also included. Fish were daily observed to record the potential symptoms of infection. Every three days internal organs were collected from 10 fish from each tank to isolate virus on cell lines and to confirmed its presence in tissue by Real-Time TaqMan RT-PCR method. Virus was isolated form cells from third day after infection to 18th dpi. The highest CPE and concentration of viral RNA was observed in the samples from RT and in hybrid RxB. The most susceptible to infection of HIRRV were RT and hybrid RxB. Only in these species was observed low mortality (6%) between 6 and 12 dpi. Observed clinical signs it was haemorrhages in muscles and internal organs, lethargy and abnormal swimming and skin darkening. In the samples collected from BT and BxA virus was isolated in a low level for a short time.

IPNV PERSISTENTLY INFECTED CELLS INDUCE PROTECTION IN NAIVE CELLS AGAINST VHSV. A CO-CULTURE SYSTEM STUDY

S. TORRES, R. PARREÑO*, L. ALMAGRO, A. ESTEPA, L. PEREZ

Instituto de Biología Molecular y Celular (IBMC-UMH), Universidad Miguel Hernández, Elche, Spain

Infectious pancreatic necrosis (IPN) is a serious viral disease of salmonid fish. IPN virus, the causative agent of the disease is able to establish persistent infections both in vivo and in vitro. We have previously characterized an IPNV-pesistently-infected fathead minnow EPC cell line (EPC^{IPNV}) which displayed resistance to superinfection with heterologous viruses such as viral hemorrhagic septicemia virus (VHSV). Although cytopathic effect and cell mortality caused by VHSV is prevented in the EPC^{IPNV} cells, it was found that VHSV is able to replicate to a certain degree in the IPNV carrier culture, as shown by the detection of VHSV-induced cell fusion (syncytia formation). To analyze in detail the induction of the antiviral state, co-culture systems were set up where the effector (IPNV-infected) cells and the target (naïve) cells were cultured separately and later put in contact by sharing the growth medium. By this method any antiviral factor secreted by the effector cells would reach the target naïve cells. It was found that EPC cells that have been exposed either to EPC^{IPNV} cells or to polyI:C-treated EPC cells became protected against VHSV challenge. Similar results were obtained in a *Transwell*® co-culture assay, where the effector (EPC^{IPNV}) cells are maintained on inserts that can be placed on top of culture plate wells seeded with the target (EPC) cells. Real-time RT-PCR measurements of interferon (ifn) and mx gene expression in effector and target cells suggest a correlation between an increased cell innate immune response and the induction of the antiviral state.

SURVEILLANCE STUDIES OF LYMPHOCYSTIS DISEASE VIRUS IN FARMED GILTHEAD SEABREAM (*SPARUS AURATA*) BY REAL-TIME PCR

E.J. VALVERDE, A.M. LABELLA, R. LEIVA, J.J. BORREGO, D. CASTRO*

Universidad de Málaga, Málaga, Spain

Lymphocystis disease (LCD) is the main viral infection reported to affect cultured gilthead seabream (*Sparus aurata*) in Southern Atlantic and Mediterranean aquaculture. Its etiological agent is the Lymphocystis disease virus (LCDV), a member of the family *Iridoviridae* (genus *Lymphocystivirus*). The only adequate measures for LCD prevention in the aquaculture systems are general prophylactic practices, such as the control of fish to be introduced in the farm facilities in order to detect carrier fish. These animals may pose a risk for the introduction of LCDV in fish farms, as direct contact between fish specimens is considered the main route of LCDV spreading. More recently, asymptomatic carrier breeders, as well as virus contaminated-live food, have been involved in LCDV transmission to fish larvae.
The detection of subclinical viral infections in carrier fish requires the use of sensitive diagnostic methods. In this context, the objective of this study was to establish the applicability of a real-time PCR assay for LCDV diagnosis in surveillance studies. In addition, the assay has been evaluated with samples from a gilthead seabream hatchery, in order to prove its utility to trace the origin of LCDV in fish farms. Juvenile fish were collected at four farms with different background regarding to LCD. LCDV was detected in all farms, and 30 to 100% of fish were identified as LCDV-infected. Estimated viral load in caudal fin of asymptomatic fish was two to five orders of magnitude lower than in diseased fish. Carrier fish were also identified in the broodstock from a farm with LCD records by analysing caudal fin samples by qPCR. In this farm, the q-PCR assay developed in this study allowed the quantitative detection of LCDV in all samples collected in the hatchery, including fertilized eggs, larvae and fingerlings, and also rotifer cultures and artemia metanauplii and cysts used for larval rearing.

This study has been supported by a project from the Spanish Government, co-funded by the FEDER, granted to D. Castro (AGL2010-17880) and a project from Junta de Andalucía (P12-RNM-2261) granted to J.J. Borrego.

VIRAL LOAD OF LYMPHOCYSTIVIRUS IN TARGET AND NON-TARGET TISSUES OF NATUALLY INFECTED GILTHEAD SEABREAM (*SPARUS AURATA*)

E. VOLPE[1], A.C.D.A.S. PINHEIRO[1], M. MOSCATO[2], M. GALEOTTI[3], S. PROSPERI[1], S. CIULLI[1]*

[1]*Alma Mater Studiorum, University of Bologna*
[2]*Panittica Pugliese, Brindisi, Italy*
[3]*University of Udine, Italy*

Lymphocystis disease virus (LCDV), (*Lymphocystivirus*, *Iridoviridae)* is the causative agent of lymphocystis, a chronic self-limiting disease described worldwide in over 125 species of fish. In the Mediterranean area, gilthead seabream (*Sparus aurata*) is the most affected species. *Lymphocystivirus* infection causes single or clustered tumour-like nodules that are localized to the skin and fins. Although lymphocystis is frequently benign, it may be responsible for significant economic losses related to poor growth rate, non-marketability of injured fish and secondary bacterial infections.
Recent surveys have shown a systemic spread of the LCDV and a persistent infection in fish after recovery. The limited availability of effective techniques for the diagnosis and study of *Lymphocystivirus*, due to their poor and difficult cultivability *in vitro*, has long reduced the possibility to study this infection. The recent development of a real-time PCR suitable for the detection and quantitation of the LCDV DNA genome in tissues of clearly or hidden infected subjects, represents a valuable new tool for pathogenesis study of lymphocystis.
The presence of viral DNA was investigated in target (pectoral and caudal fins) and non-target (spleen, brain, eye) tissues of 12 naturally infected gilthead seabream. The infected fish were ranked on the basis of presence of typical lesions (4 subjects), scarring due to the regressive phase of the disease (4 subjects) and absence of any clinical alterations (4 subjects).
The viral DNA was detected in all tested fish and tissues. Different viral loads were detected in target and non-target tissues showing statistically higher amount of viral DNA in pectoral and caudal fins (10^6-10^7 viral DNA copies/μl) than that detected in internal organs (10^3-10^5 viral DNA copies/μl).
The distribution of the viral genome showed a similar pattern regardless of the clinical stage of the fish. In the caudal-pectoral fins and spleen viral loads were detected in decreasing, but not statistically significant, values in diseased, in regression and recovered subjects, respectively.
The viral DNA was always detected in recovered fish with variable values in different organs (10^2-10^7 viral DNA copies/μl) showing a persistent infection after symptom remission.
The present study confirmed systemic and persistent infection adding a quantitative analysis.

DETECTION OF CARP EDEMA-LIKE VIRUS IN ARCHIVE DNA AND TISSUE SAMPLED FROM DISEASE OUTBREAKS IN COMMON CARP (*CYPRINUS CARPIO*) IN THE UK AND THE NETHERLANDS: A LINK WITH SPRING CARP MORTALITY SYNDROME

K. WAY[1]*, P. MARTIN[1], A. READING[2], C. WILLIAMS[2], M. ENGELSMA[3], O. HAENEN[3], D. STONE[1]

[1]*Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset, UK*
[2]*Environment Agency, Brampton, Cambridgeshire, UK*
[3]*Central Veterinary Institute (Wageningen UR), Lelystad, The Netherlands*

The pox virus that is the disease agent of koi sleepy disease (KSD) was originally described in Japan in the 1970's as a viral oedema of juvenile koi carp (CEV). The virus was also shown to affect adult koi and cause severe damage to gill lamellae, leading to hypoxia, lethargy (sleepy behaviour) and death from anoxia. Losses from CEV were seen in spring and autumn, over a temperature range of 15 – 25°C, and mortalities reached 80%.

In Europe, outbreaks of KSD and PCR detections of CEV-like virus were reported from 2009 and, by early 2015, seven EU member states had reported multiple KSD outbreaks in imported koi. CEV-like virus was confirmed, in all cases, using improved PCR assays. Then in 2012, a CEV-like virus was detected for the first time in common carp, displaying KSD-signs, at a fishery in England, then at other English fishery sites later in 2012 and 2013/2014, and in common carp fisheries in other EU member states.

Phylogenetic analysis of the amplification products, from The Netherlands and UK detections, revealed two main lineages of CEV-like virus. All the detections obtained from koi are contained in one lineage and some show close phylogenetic similarity to the original Japanese CEV. All of the detections from common carp are contained in a second lineage.

Analysis of archive DNA samples from disease outbreaks in common carp indicate that CEV-like virus has been present in the UK and The Netherlands since 2004. Interestingly, the disease outbreaks in common carp, displaying KSD-signs, are often seen at lower water temperatures (6-10°C) than those in koi. This prompted the speculation that the CEV-like agent may be associated with Spring Carp Mortality Syndrome (SCMS), a disease syndrome, of unknown aetiology, that was first reported in England in the 1980's.

CEV-like virus was detected in a high proportion of archive DNA samples and at high levels, by PCR and qPCR, from suspected SCMS cases in England. Currently, archive paraffin-wax embedded tissue blocks, from SCMS investigations conducted over 15 years ago, are examined for presence of CEV-like virus. The results of this investigation will be presented and the impact for carp culture in Europe will be discussed.

STUDY ON VIRAL NERVOUS NECROSIS (VNN) DISEASE IN *MUGILIDAE* FISH *(LIZA KLUNZINGERI & MUGIL CEPHALOUS)* OF OMAN SEA AND PERSIAN GULF USING HISTOPATHOLOGY, IMMUNOFLUORESCENCE ANTIBODY TEST (FAT) AND HEMATOLOGY METHODS

M.E.J. ZORRIEHZAHRA[1]*, V. SARABANDI[2], A. GHAHGHAEI[2], G.-R. MOTALLEB[3], M. GHASEMI[4], M.R. MEHRABI[1], K. RADKHAH[1], O. KOOHKAN[5], I. SHARIFPOUR[1]

[1]*Iranian Fisheries Research Organization, (IFRO), Tehran, I.R.Iran*
[2]*University of Sistan & Baluchestan, Zabol, I.R. Iran*
[3]*University of Zabol, Zabol, I.R. Iran*
[4]*Inland Water Aquaculture Research Center, Bandar Anzali, I.R. Iran*
[5]*Chabahar Maritime University, Chabahar, I.R. Iran*

Viral Nervous Necrosis (VNN) disease is a lethal emerging disease; especially in marine fishes by the economic loses. In this study about 300 samples were collected from Mugilidae fish family in southern beach of Iran. The range of length and weight of fish was between 8 to 18 cm and 15 to 200 gr, respectively. Target tissues such as eye and brain were collected for diagnostic tests. Through a special questionnaire, Ecological characteristics such as Physico-chemical properties of the fishing location and biometry details, clinical signs and autopsy findings were recorded which were used in the final analysis. In order to investigate haematological changes, Blood sampling was done before the death of the fish samples. Also, Immunofluorescence antibody test (FAT) was done against Viral Nervous Necrosis (VNN) virus antigen. By survey of obtained results, moribund Maid fish (*Liza klunzingeri*) revealed clinical signs such as changing in body coloration, abnormal swimming behavior, belly up, disorientation, ventral and operculum haemorrhage but in *Mugil cephalous* no clinical signs of disease were observed on none of the fish samples. FAT findings were negative and showed no viral antigen and apparently all slides were without any antibody- antigen complex. Only one slide seems positive that it was not sufficient findings to definite Viral Nervous Necrosis (VNN). In Pathology specimens, both of them revealed evidence of inflammation, hyperemia and bleeding and cerebral vasodilatation, accumulation of macrophages (MMC), also cell necrosis and severe vacuolation were seen. In haematological studies, WBC, Hb, Hct and RBC count in infected group were significantly different from control group but MCV, MCH and MCHC had no significant differences. In pathogenicity test challenging with the brain-homogenate was carried out on Guppy (*Poecilia reticulata*) as susceptible fish. A few challenged fishes showed the same clinical and behavioral signs with infected Maids, and mortality was low. In some fishes abnormal and neural behaviors were observed and some of them showed ventral swelling. These results showed that VNN disease could be one of the important probably reasons for recent acute mortality in *L. klunzingeri* and it would be approved with comprehensive studies and more investigations in future cases.

STUDY ON THE EFFECTS OF VIRAL NERVOUS NECROSIS (VNN) DISEASE ON SOME BLOOD BIOCHEMICAL PARAMETERS IN GOLDEN GREY MULLET (*LIZA AURATA*) IN SOUTHERN COASTAL WATERS OF CASPIAN SEA

M.E.J. ZORRIEHZAHRA[1]*, A.A SAEIDI[2], M. GHIASI[2], M.R. MEHRABI[1], R. POURGHOLAM[2], M. BINAEII[2]

[1]*Iranian Fisheries Research Organization, (IFRO), Tehran, I.R. Iran*
[2]*Caspian Sea Ecology Research Centre, Sari, I.R. Iran*

Viral Nervous Necrosis (VNN) as worldwide emerging disease was reported as first time in *Liza aurata* in Caspian Sea in 2004. In this survey, the effects of Viral Nervous Necrosis (VNN) diseases on some of the blood Biochemical parameters were studied in Golden grey mullet (*Liza aurata*) at Southern part of Caspian Sea. About 128 fish sample were captured from Babolsar, Fereydonkenar, Mahmoud Abad, Noor and Tonekabon stations. Moribund fish samples were selected that revealed some pathognomonic clinical signs such as abdominal distension change in swimming behaviour, belly up and darkening in skin. Samples biometry calculations were done and fish ages were measured according to reading scales operation. Blood sample were collected from caudal vein and essential serum was isolated immediately from whole fish blood. Biochemical factors of serum, Total Protein, Albumin, Complement elements such as C3, C4 and IgM, were measured using standard methods and then compared with biochemical factors on health fish as control group. Excel 2010 software was used to calculating the serum parameters and drawing the related graphs. Statistical analysis was done by One-Way ANOVA and SPSS (Ver.18) software. Results revealed that all measured factors (exception C3) in affected fish with pathognomonic clinical signs of VNN were significantly lower than health fish. C3 in health fish were higher than fish with clinical symptoms but there were no significant differences between them. Also, 60% of samples were two years old and most of them were captured from Babolsar station. Biochemical parameters were studied in other diseased species of fish and their results shown that VNN diseases could be reduce the Total protein, Albumin and IgM that all of the mentioned decrement would be specific indicator for immunity deficiency in cited degenerative disease and could be introduce VNN disease as a new protein catabolism disorder in marine fish.

STUDY ON HAEMATOLOGICAL FACTORS OF GOLDEN GREY MULLET (*LIZA AURATUS*) SUSPECTED TO VIRAL NERVOUS NECROSIS (VNN) DISEASES CAPTURED IN SOUTHERN COASTAL WATERS OF CASPIAN SEA

M.E.J. ZORRIEHZAHRA[1]*, M. GHIASI[2], A.A SAEIDI[2], R. POURGHOLAM[2], M.R. MEHRABI[1], M. BINAEII[2]

[1]*Iranian Fisheries Research Organization, (IFRO), Tehran, I.R. Iran*
[2]*Caspian Sea Ecology Research Centre, Sari, I.R. Iran*

Viral Nervous Necrosis (VNN) as an emerging disease has been considered in Caspian Sea since 2003. This study was designed to investigate the interaction effects of Viral Nervous Necrosis (VNN) disease on the blood parameters of Golden grey mullet (*Liza auratus*) in Southern coast of the Caspian Sea. About100 pieces of infected Golden grey mullet with emphasis on pathognomonic clinical signs of Viral Nervous Necrosis (VNN) disease were captured in sizes 20 up to 30 cm from the start of the fishing season of bony fish in the Caspian Sea in September 2012; the station branches were located in Noor, Mahmoud Abad, Babolsar, Fereydonkenar and Tonekabone. Samples were done with using of gill net coastal fisheries. All fish samples were transferred to haematology laboratory of Caspian Sea Ecology Research Center, and then biometric information were recorded and blood samples were taken from fish caudal vein. Haematological parameters including Red and White blood cells count, Haematocrit, Haemoglobin , Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Haemoglobin (MCHC), were determined that the two groups of fish consist of one year ,and two years, no significant differences were observed between the examined blood parameters ($P \geq 0/05$) and Viral Nervous Necrosis disease and Golden grey mullet between habitats, there is a significant correlation ($P \leq 0/05$), and also VNN could be affected on the amount of blood or haematology factors. Meanwhile, a significant correlation ($P \leq 0/05$) was observed between VNN and location of affected fishes in the Caspian Sea. Also, Normocytic Hypochromic Anaemia disorder was diagnosed in Babolsar and Tonekabone areas in affected fish. Mentioned anaemia was recorded from chronic dystrophy diseases that could be indicated long degenerative disorder in affected fish in the Caspian Sea.

SCREENING OF PROBIOTIC BACTERIA FOR THE CONTROL OF *STREPTOCOCCUS INIAE* AND *EDWARDSIELLA PISCICIDA* IN TILAPIA (*OREOCHROMIS NILOTICUS,* LINNEUS 1758)

M. ETYEMEZ[1]*, J.L. BALCAZAR[2], İ. CENGİZLER[1], Ş. TABAKOĞLU[1]

[1]*Department of Aquaculture and Fish Diseases, Faculty of Fisheries, University of Cukurova, Adana 01330, Turkey*
[2]*Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Girona 17003, Spain*

The use of probiotics is gaining interest in the aquaculture industry as an environmentally friendly management alternative to the use of antibiotics and other antimicrobials for disease prevention. The aim of the present study was therefore to isolate potential probiotic bacteria from the intestinal mucus of tilapia, and to study their antagonistic activities against two pathogenic strains (*Streptococcus iniae* and *Edwardsiella piscicida*). One hundred and twenty bacterial strains were isolated and screened for antagonistic activity. Based on the results of the *in vitro* antagonism tests, the number of potential probiotic strains was reduced to five strains, which were further identified by 16S rRNA gene sequence analysis. Bacteria belonging to the genus *Bacillus* showed inhibitory activity against the selected pathogens. It was found that *Bacillus subtilis* subsp. *inaquosorum, Bacillus sonorensis, Bacillus endophyticus* and *Bacillus flexus* inhibit the growth of *Streptococcus iniae,* whereas *Bacillus mojavensis* inhibit the growth of both *Streptococcus iniae* and *Edwardsiella piscicida.* According to our results, these bacterial strains should be further studied to explore their probiotic effects under *in vivo* conditions.

IMMUNE STIMULATION IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*) LARVAE BY POLY-ß-HYDROXYBUTYRATE ADMINISTRATION

A. FRANKE[1]*, O. ROTH[2], P. DE SCHRYVER[2], C. CLEMMESEN[1]

[1]*GEOMAR Helmholtz Center for Ocean Research Kiel, Kiel, Germany*
[2]*Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belgium*

Global aquaculture production is growing rapidly, however, the industry is facing great challenges such as high mortality rates during early life stages. The lack of a fully mature immune system makes larvae highly sensitive to infectious diseases. One of the most common pathogenic bacteria in larviculture is *Vibrio anguillarum* causing worldwide severe economic losses. Due to the development of antibiotic resistances, the establishment of alternative methods to prevent and control diseases, ensure efficient growth and reach maximal survival rates is mandatory to optimize aquaculture productivity. A promising solution might be the early activation of the immature immune system of fish larvae by administration of immunostimulants as nutritional supplements. In our study we assessed the potential immunomodulatory effect of poly-ß-hydroxybutyrate (PHB) in European sea bass (*Dicentrarchus labrax*) larvae. PHB is a bacterial energy storage compound which may have a potential application as an immunostimulant in fish culture. In our experiment we used rotifers as live carriers to feed PHB-accumulating bacteria (*Alcaligenes eutrophus*) to first-feeding European sea bass larvae over a period of 14 days. To estimate the immediate impact of PHB, larval mortality rates were monitored daily during the course of the experiment. In order to assess the disease resistance of the larvae, survival rates after bath challenge with *Vibrio anguillarum* after 3 and 14 days of PHB treatment and 7 days post PHB treatment were monitored. Furthermore, we determined gene expression profiles for immune genes as well as metabolism- and stress-related genes. Results will be discussed in respect to the use of PHB in fish hatcheries and its effect on the immune system of first feeding sea bass.

SACCHAROMYCES CEREVISIAE VAR. *BOULARDII* AS PROTECTION OF INTESTINAL MUCOSA AGAINST *VIBRIO ANGUILLARUM*

M. MANCUSO[1]*, L. CACCAMO[1], C. PEDÀ, A. PERDICHIZZI, L. GENOVESE[1], G. MARICCHIOLO[1]

[1]*Institute for Coastal Marine Environment, CNR- Messina, Italy*

S. cerevisiae boulardii (LSB, LEVUCELL SB20®) was used as probiotic in order to evaluate the potential protective effect on intestinal mucosa against one of the major fish pathogen *Vibrio anguillarum*, during an experimental challenge on gilthead sea bream *(Sparus aurata)*. The experiment was carried out at Aquaculture Experimental Plant of IAMC-Messina in the indoor tanks (1,4 m^3) equipped with a sea water flow-through system. 36 fish were divided into three diet treatments (12 fish/group). The control group G1 was fed with a basal diet, probiotic free, (LSB0), while the experimental groups: G2 (LSB 100) and G3 (LSB 300) were fed with the basal diet enriched with the yeast at the concentration of 5,4x 10^5 and 1,08x10^5 CFU/g diet respectively. The feeding protocol was followed for 21 days, then fish were challenged with *V. anguillarum* O1 strain at the density of 3x10^4cell/ml. *S. aurata* were intraperitoneally injected with 0.1 ml of bacterial suspension. Seven days after challenge, all fish were sacrificed previous euthanasia. Samples were taken to perform histological and bacteriological assays. The autopsy showed that in G1 and in G2 groups, the intestine appeared slightly bleeding, while in G3 group no bleeding was noticed. G1 and G2 groups presented massive hemorrhagic necrotic enteritis in the intestinal lumen; in contrast, the intestinal cells of the G3 group appeared to be protected from the attack of *V. anguillarum.* Levucell SB20®, used in an appropriate dose, has been shown to exert a protective effect on the intestinal mucosa; therefore its use is recommended in the diet of *S. aurata.*

EFFECTS OF A DIETARY MESOPHILIC PROBIOTIC *LACTOBACILLUS RHAMNOSUS* ON PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT

N. TOPIĆ POPOVIĆ[1]*, I. STRUNJAK-PEROVIĆ[1], R. SAUERBORN-KLOBUČAR[1], J. BARIŠIĆ[1], M. JADAN[1], S. KAZAZIĆ[1], A. PREVENDAR CRNIĆ[2], J. ŠURAN[2], B. BEER LJUBIĆ[2], V. MATIJATKO[2], R. ČOŽ-RAKOVAC[1]

[1]*Ruđer Bošković Institute, Zagreb, Croatia*
[2]*University of Zagreb, Veterinary Faculty, Zagreb, Croatia*

Lactobacillus rhamnosus was mixed in the trout diet with peptone water to attain two diet groups with concentrations of 10^7 (G1) and 10^8 CFU (G2). The G3 group was fed with feed sprayed only with peptone water. Fish were fed for six weeks and sampled at the beginning and end of the trial, and three weeks after the feeding trial. Before the trial, fish had no detectable lactic acid bacteria in the intestines nor in the fecal contents. The numbers of viable lactobacilli from fecal contents increased in G1 and G2 from below detection limits (<10 CFU/mL) to levels between 2 x 10^2 and 4 x 10^5 CFU/mL (G1), between 4.2 x 10^2 and 8 x 10^2 CFU/mL (G2) by the end of the trial. Even G3 demonstrated viable lactobacilli between neglectable to 0.6 x 10^4 CFU/mL.
The feeding with supplemented probiont changed the resident microbiota in fish from *Vibrio fluvials, Aeromonas hydrophila, Serratia fonticola* before the trial, to *V. fluvialis*, *Burkholderia cepacia,* non-fermenter species, and *Pasteurellaceae* by the end of the trial. For tested plasma metabolites, no significance was found in feeding categories between lower and higher *L. rhamnosus* concentrations, implying that for the tested variables, a difference in probiont concentration was not as important as duration of treatment in comparison to withdrawal period. A decrease in SOD was noted in the control and both G1 and G2 withdrawal groups, both GSH-Px and PON 1 increased after the withdrawal phase. A significantly higher liver TBARS level was observed in G2 group six weeks after the start of the feeding trial with supplemented diet (G2). Plasma glucose, urea, creatinine, total proteins, albumins, triglyceride, cholesterol, SOD, GSH-Px stood out with a potential to indicate to the impact of probiont upon the overall health status of rainbow trout.

CHARACTERIZATION OF *CRYPTOSPORIDIUM SCOPHTHALMI* – LIKE ORGANISM FROM TURBOT (S*COPHTHALMUS MAXIMUS*) USING HISTOLOGICAL AND MOLECULAR TOOLS

J. COSTA[1], C. CRUZ[1, 2], J.C. EIRAS[1, 2], A. SARAIVA[1, 2 *]

[1]*CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, Porto, Portugal*
2Faculty of Sciences, University of Porto, Porto, Portugal

The Apicomplexa *Cryptosporidium sp.* is the cause of numerous waterborne, food-borne and day-care outbreaks of diarrheal disease worldwide. Currently little is known about the prevalence and geographic distribution of *Cryptosporidium* isolates that infect fish. The first report of a *Cryptosporidium* species in fish was in 1981, in a tropical marine fish (*Naso lituratus*), and *Cryptosporidium nasorum* was subsequently proposed as a species in 1984. More recently, *Cryptosporidium molnari* was isolated from two marine species, gilthead seabream (*Sparus auratus*) and European seabass (*Dicentrarchus labrax*), and *Cryptosporidium scophthalmi* was isolated in the intestinal and stomach epithelia of turbot (*Scophthalmus maximus*). However, molecular characterization of these species is still incomplete, and needs to be addressed to properly establish the genetic relationship to other *Cryptosporidium* species.
In the present study we performed histological, genetic and phylogenetic analysis of a *Crypstosporidium scophthalmi* – like isolate from turbot (*S. maximus*). Fish (n=30) were sampled from a fish farm located in the North of Portugal. Intestine was removed and a portion was stored in 70 % ethanol for molecular analysis. For histological analysis, a portion of intestine was fixed in 10% neutral formalin for 48 hours and stored in 70% ethanol until processing. Molecular identification was performed by sequencing of the 18S rRNA and actin genes. Histological analysis were conducted using standard techniques and tissues were stained with haematoxylin and eosin. A *Cryptosporidium scophthalmi*-like organism was observed in intestinal histological sections, and molecular analysis revealed that this parasite was genetically closer with other *Cryptosporidium* isolates previously identified in other fish species.

This work was partially funded by the Project AQUAIMPROV (reference NORTE-07-0124-FEDER-000038), co-financed by the North Portugal Regional Operational Programme (ON.2 – O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF).

PREVALENCE OF *SAPROLEGNIA* SPECIES COLLECTED FROM FISH FARMS IN SCOTLAND

M.J. BECKMANN[1]*, C.J. SECOMBES[2], P. VAN WEST[1]

[1]*Oomycete Laboratory, University of Aberdeen, Aberdeen, UK*
[2]*Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, UK*

Fish farms and hatcheries worldwide suffer heavy losses every year during the fresh water stages of salmon production due to infection by filamentous oomycete pathogens. Several species of oomyectes have been found to be pathogenic to fishes and their eggs. Among these *Saprolegnia diclina* and *Saprolegnia parasitica* belonging to the order of Saprolegniales are considered the most notorious oomycete parasites to greatly impact fish production in Scotland. *S. diclina* is commonly found on infected eggs while *S. parasitica* causes Saprolegniosis on fish raised in net-pen farms in Scottish lochs. Saprolegniosis is typically characterised by cotton-wool like, white growth of mycelia on the fish skin, dorsal and caudal fins and gills. We collected approximately 250 isolates of *Saprolegnia sp.* and other oomycetes between 2012 and 2015 most originating from infected salmon or tissue samples of uninfected salmon, as well as baited water samples from around Scottish fish farms and hatcheries. The isolates were identified by phylogenetic analysis of the ITS region and genetically characterised by RAPD-PCR. The majority of the isolates collected from hatcheries and fish farms belong to one of the two species, *S. parasitica* or *S. diclina*. Only one isolate of *S. diclina* was collected from a loch farm, while *S. parasitica* was equally found in hatcheries as well as loch sites. Despite samples originating from widely separated geographical locations we found currently only 3 distinct genotypes of *S. diclina* with most isolates belonging to only 2 of these distinct genotypes. At this stage we identified 8 distinct genotypes of *S. parasitica.* Most locations seem to share closely related genotypes of *S. parasitica* but can also harbour distinctively different genotypes not found at another site. Our findings show that virulent clonal isolates are prevalent at most tested sites.

DEVELOPMENT OF NON-LETHAL SAMPLING TECHNIQUES TO INVESTIGATE ATLANTIC SALMON (*SALMO SALAR*) IMMUNE RESPONSES TO THE ECTOPARASITE *NEOPARAMOEBA PERURANS*, THE ETIOLOGICAL AGENT OF AMOEBIC GILL DISEASE (AGD)

R. CHANCE*, Z. ALLCOCK, C. COLLINS, B. COLLET, C. SECOMBES

Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, U.K.
Marine Laboratory, Marine Scotland Science, Aberdeen, UK

Current methodology for investigating pathogen-induced immune responses in fish relies upon lethal sampling of different individuals at set time points. By following a non-lethal methodology, there is a potential 80-90% reduction in experimental fish needed for ectoparasite challenge experiments. Non-lethal sampling will also provide more conclusive results by improving the linking of response dynamics and infection outcome; a reduction in host, parasitic load and localised response variability; prevention of masking of patterns; and statistical robustness. The pathogen selected to act as a model for the design of this methodology is *Neoparamoeba perurans*, the etiological agent of amoebic gill disease (AGD), a globally important ectoparasite of farmed fish. During non-lethal sampling, amoebae will be repeatedly exposed to anaesthetic. Initial work has focused upon assessing whether repeated exposure of fish anaesthetics affected the growth rate or viability of *N. perurans*. In order to simulate this, *in vitro* cultures of *N. perurans* were exposed to anaesthetic for 20 min every four days for 28 days. Prior to exposure, viability was assessed with a Neutral Red assay and growth rate of attached amoebae was determined by counts of amoebae in photographs (five fields of view) taken under a microscope. Results await statistical analysis at time of abstract submission, but suggest that metomidate and MS-222 both had negative effects on viability and growth. Current work will focus upon novel *in vivo* assessment of fish gill health during multiple exposures to anaesthetic, non-lethal sampling of blood and gill mucus during AGD challenge to investigate systemic and localised immune responses.

COMPARATIVE MORPHOLOGICAL STUDY OF GREATER AMBERJACK (*SERIOLA DUMERILI*) SKIN BETWEEN FISH INFECTED AND NON INFECTED WITH MONOGENEA *NEOBENEDENIA GIRELLAE*

A. FERNÁNDEZ-MONTERO*, M.J. CABALLERO, S. TORRECILLAS, M.S. IZQUIERDO, D. MONTERO

Grupo de investigación en acuicultura (GIA), University of Las Palmas de Gran Canaria, Parque Científico-Tecnologico Marino, Muelle de Taliarte s/n, Telde, Las Palmas, Canary Islands, Spain

The greater amberjack (*Seriola dumerili*, Risso 1810) is a emerging species for aquaculture, and is consider as a fast-growing fish. However, the production of this species have an important bottleneck in the on-growing period, since this fish species has been reported to be very susceptible to monogenea infection, and is specially susceptible to *Neobenedenia girellae.* The objective of the present study was to compare morphology of skin between fish infected and non infected with this parasite species.

Samples of skin of different individual (around 700 g body weight) were collected and prepared for the histological analyses in buffered formalin at 4%. The histopathological evaluation was made with the samples that were previously stained with a hematoxylin-eosin staining protocol.

Usually, infection with *N. girellae* is associated with wounds and secondary infections related with scratching behaviour of infected fish, leading to high mortality rate. From parasitized animals, a local immune response could be observed at the skin level, especially in the adhesion regions of the parasite to the host. *N. girellae* like other monogenea, present two adhesion structures named prohaptor and opisthaptor. The opisthaptor possess two central hooks and penetrates to dermis inducing a lymphoid infiltrate around the insertion site. A comparative study between infected and non-infected fish is presented.

METHODS OF IDENTIFYING VACCINE CANDIDATES IN THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*) USING A PROTEOMICS APPROACH

S. HAMILTON[1]*, S. MONAGHAN[2], C. MCNAIR[2], N. INGLIS[1], K. MCLEAN[1], W. ROY[2], H. MCDONALD, A. ADAMS[2], K. THOMPSON[2], R. RICHARDS[2], T. KANELLOS[3], D. ASPER[3], J.E. BRON[2], D.P. KNOX[1]

[1]*Moredun Research Institute, Midlothian, Scotland*
[2]*Institute of Aquaculture, Stirling, Scotland*
[3]*Zoetis, Kalamazoo, United States*

Background: The salmon industry in Scotland accounts for £1bn in contributions to the United Kingdom economy. The cost associated with controlling the sea louse parasite *Lepeophtheirus salmonis* (*L. salmonis*) is estimated at £33m per annum. There is therefore a need for a novel, effective and environmentally friendly solution to reduce *L. salmonis* numbers in farmed salmon.
Aim: Our aim is to produce a vaccine which will reduce sea louse numbers in farmed salmon and consequently contribute to sustainable protein production in the UK.
Materials and Methods: Secretion assays were performed with different life stages of *L. salmonis*. The spectral data generated from LC-ESI-MS/MS was analysed to reveal protein profiles. Proteins which were selected as being strong candidates were subjected to further investigation using three approaches: 1. Affinity chromatography, 2. Recombinant technology and 3. Protein fractionation. Vaccine trials were conducted in Atlantic salmon (*S. salar*) using these methods.
Results: Proteases were found to be present in the secretions of *L. salmonis*. Protein fractionation revealed an increase in relative abundance of target proteases. Mass spectrometry data from samples subjected to affinity chromatography revealed an enrichment of target proteins. Recombinant proteins were used to inoculate 150 salmon with results pending.
Discussion: Results from the vaccine trial will be collated and presented. Further trials will be optimised pending current trial results. From the mass spectrometry data collected, a cocktail recombinant vaccine will be produced and trialled in salmon.

HISTOPATHOLOGY OF MICROSPORIDIAN INFECTING NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FROM CAGE FISH FARMING ON HYDROELECTRIC RESERVOIRS IN BRAZIL

M.V. RODRIGUES*, C.J. FRANCISCO, J.P. ARAÚJO JÚNIOR, M.C.C. DE SOUZA, M. BONGIOVANI, F. HENRIQUE, G.S. DAVID, R.J. DA SILVA

Univ. Estadual Paulista (UNESP), Distrito de Rubião Júnior, São Paulo, Brazil

Microsporidians are included in a very diverse group of organisms with currently 143 genera and 1200 named species. Of these, 156 microsporidian species were recorded infecting fishes. These pathogens can cause economically important diseases in insects, fishes, and mammals.These obligate intracellular pathogens are true eukaryotes, but they also display molecular and cytological characteristics reminiscent of prokaryotes, which are characterized by their unique mechanism to infect host cells. Fish microsporidian are embedded directly in the cytoplasm of the host cell which they actually destroy or induce enormous cell hypertrophy. They transform it into a special formation, the xenoma, in which the developing parasite and host cell represent a physiologically integrated whole. The aim of this research was to describe histopathological lesions observed in Nile tilapia (*Oreochromis niloticus*) sampled on cage fish farming in Brazil. All the organs (gills, heart, liver, spleen, stomach, gut, gall blader, kidney, muscle, and brain) were taken (1 cm^3) and fixed in formaldehyde 10%. The tissues fixed were embedded in paraffin using standard histological procedures. Paraffin sections (4 μm) were stained with H&E. It was observed in the histopathology xenoma in the gills and gut, hypertrophy and lamellar fusion, inflammatory response in all tissues, presence of intra-nuclear basophilic structures in leukocytes suggesting microsporidian infection, and presence of eosinophils. As previously described, this disease can cause xenoma formation, inclusion intra-nuclear in leukocyte, hypertrophy, proliferation and dispersed of infected hematopoietic cells, as observed in this research. Based on histopathology finds, we conclude that these lesions could be caused by a microsporidian, which needs a confirmatory test like molecular techniques, *in situ* hybridization or electron microscopy.

SUSCEPTIBILITY OF NON-NATIVE HOST *PROTERORHINUS SEMILUNARIS* TO LOCAL PARASITES IN THE DYJE RIVER

M. ONDRAČKOVÁ[1], I. HUDCOVÁ[1], I. DYKOVÁ[2], M. DÁVIDOVÁ[2]*, P. JURAJDA[1]

[1]*Institute of Vertebrate Biology ASCR, v.v.i., Brno, Czech Republic*
[2]*Faculty of Science, Masaryk University Brno, Brno, Czech Republic*

Tubenose goby (*Proterorhinus semilunaris*) has recently spread beyond its Ponto-Caspian range. After its introduction by anglers into the Musov Reservoir of the Dyje River in 1994, this fish species rapidly expanded down the river and established abundant populations. In this study, we investigated the level of local parasite acquisition by non-native tubenose goby in the Dyje River (Danube basin, Black Sea drainage) sixteen years after the host introduction. Out of the twelve parasite species found in tubenose gobies, only one species (*Gyrodactylus proterorhini*) was co-introduced with the host. High susceptibility to local parasites was documented for larvae of unionid bivalves, glochidia. Maximum prevalence was found in glochidia of *Anodonta anatin*a, being observed in all fish examined; glochidia of *Unio tumidus* showed high intensities of infection with almost 100 parasites per fish. High intensities of infection (over 150 worms per host) showed also the nematode *Schulmanella petruschewski* located mainly in the fish liver. Histopathological examination of infected liver demonstrated extensive pathological changes. Liver tissue atrophy was apparent in the proximity of adult nematodes and necrosis was observed around the clusters of released eggs. Proliferative inflammation manifested by connective tissue increase at the expense of hepatocytes and by structure alteration of the whole organ. High intensities of infection led to the reduction of the host liver function. Vulnerability to at least three parasite species in the new area indicates that tubenose goby may have important implications for local parasite population dynamics.

This work was supported by ECIP (GBP505/12/G112).

NEW CONTRIBUTIONS TO MYXOSPOREAN (MYXOZOA) FAUNA OF THE BLACK SEA FISHES – A COMPARISON OF PAST AND CURRENT STATUS

A. ÖZER[1]*, H. ÖZKAN[1], V. YURAKHNO[3]

[1]Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000 Sinop, Turkey
[2]A.O. Kovalevsky Institute of Biology of the Southern Seas, 2 Nakhimov av., 299011 Sevastopol, Russia

In this comprehensive research study, a total of 816 fish specimens belonging to 31 fish species collected from Sinop coasts of the Black Sea were investigated for myxosporean parasites in the period from February 2013 to November 2014. Gills, urinary bladder, kidney, gall bladder, intestine and gonads were examined at x400 magnification using a phase contrast microscope equipped with a digital camera and hand drawing attachment at the Faculty of Fisheries and Aquatic Sciences in Sinop, Turkey. A total of 19 myxosporean species was identified from 14 fish species; their identities and hosts were *Sphaeromyxa sevastopoli* in *Parablennius sanguinolentus*; *Myxidium parvum* in *P. tentacularis* and *Salaria pavo; M. gadi* in *Merlangius merlangus; Enteromyxum leei* in *Chromis chromis; Ortholinea gobiusi* in *Neogobius melanostomus; O. divergens* in *P. sanguinolentus; O. orientalis* in *Mullus barbatus* and *Alosa tanaica; Ceratomyxa merlangi* in *M. merlangus; Myxobolus muelleri*(?) in *Diplodus annularis; M. asymmetricus* in *P. tentacularis* and *P. Sanguinolentus; M. parvus* in *Liza saliens* and *M. barbatus; M. rotundus*(?) in *Symphodus cinereus; Myxobolus* sp1. in *N. melanostomus* and *P. tentacularis; Myxobolus* sp2. in *Gobius paganellus; Myxobolus* sp3. in *G. niger; Myxobolus* sp4. in *L. saliens; Henneguya* sp. in *P. tentacularis; Sinuolinea rebae* in *Solea solea; Polysporoplasma mugilis* in *L. saliens.* The results obtained throughout the present study were compared with 69 myxosporean species so far been reported in the Black Sea, their microhabitats and hosts. Of the 19 identified myxozoan parasite species, 3 species (*E. leei, O. orientalis, S. rebae*) were new records for the Black Sea parasite fauna as well as 11 species (*S. sevastopoli, M. parvum, E. leei, O. gobiusi, O. divergens, O. orientalis, M. asymmetricus, M. parvus, M. rotundus, S. rebae, P. mugilis*) were new records for Turkish parasite fauna. Several myxosporean species were also determined from previously not reported host species and therefore were registered for new host records. Current study also yielded 5 myxosporean species identified only at genus level from new hosts and believed to be new for science. This study is the first to provide a comprehensive and comparative data on the past and current status of the myxosporean fauna of the Black Sea fishes.

COMPARATIVE INFECTION LEVELS OF *HYSTEROTHYLACIUM ADUNCUM* (NEMATODA) IN WHITING *MERLANGIUS MERLANGUS* AT THE SOUTHERN AND NORTHERN COASTS OF THE BLACK SEA

A. ÖZER[1]*, Y. KORNYYCHUK[2], V. YURAKHNO[2], T. ÖZTÜRK[1]

[1]*Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000 Sinop, Turkey*
[2]*A.O. Kovalevsky Institute of Biology of the Southern Seas, 2 Nakhimov av., 299011 Sevastopol, Russia*

In the present study, we investigated the comparative infection levels, ecology and host-parasite interrelationships of a nematode *Hysterothylacium aduncum* (Rudolphi, 1802) in the digestive tract of whiting, *Merlangius merlangus* (L., 1758), in Turkish and Russian coasts of the Black Sea for the first time. Fish were collected throughout a period from May 2011 to March 2014 from local fishermen. A total of 690 fish specimens near Sinop, Turkey and 423 fish near Sevastopol, Russia were examined for parasites. Nematodes were determined by examining the digestive tract using a light microscope, identified and counted. Prevalence (%), mean intensity and mean abundance values were determined according to Bush *et al.* (1997). Quantitative Parasitology 3.0 software was used to calculate Sterne's exact 95% confidence limits for prevalence bootstrap 95% confidence limits for mean abundances and mean intensity. Difference in prevalence values between two fish populations collected from two sampling locations and sex was determined by exact unconditional test while difference in prevalence values between sampling seasons, age and length categories was determined by Fisher's exact test. Differences in mean abundance and intensity were performed by bootstrap two-sample *t* test. *Hysterothylacium aduncum* was the only nematode species identified in the digestive tract of Black Sea whiting *Merlangius merlangus*. Prevalence of infection, mean intensity and mean abundance values in Turkish samples were higher than those in Russian samples. Overall prevalence of infection 80.3% and 32.9%, mean intensity 11.5 and 3.1, mean abundance 18.1 and 1.0 were determined in Turkish and Russian samples, respectively. Gradual increases of infection indices in relation with increasing host length classes were determined in Turkish samples; however the situation was reversed in Russian samples. Infection indices in female and male fish at both locations were very similar; however the differences were significant between localities. Autumn and winter in Turkish samples and winter and spring in Russian samples had higher infection indices. These differences in infection levels at both localities were evaluated and presented in tables. This data is the first comparative data on *H. aduncum* infection in whiting collected from northern and southern coasts of the Black Sea.

ENDEMIC *APHANIUS DANFORDII* (PISCES: CYRINODONTIFORMES): A NEW HOST SPECIES OF *CLINOSTOMUM COMPLANATUM* METACERCARIAE (DIGENEA)

T. ÖZTÜRK[1]*, A. ÖZER[1]

[1]*Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000, Sinop, Turkey*

The toothcarp, *Aphanius danfordii* (Boulenger, 1890) is a small cyprinodontid fish and inhabits brackish water of coastal lagoons, river-mouths and freshwater habitats. It is endemic in Turkey and distributes in the drainage system of the Kızılırmak and Yeşilırmak Rivers and in the upper Seyhan River. The aim of this study was to describe the occurrence of *Clinostomum complanatum* metacercariae infecting the toothcarp collected from the Lower Kızılırmak Delta, a natural conservation area for wild life diversity including migratory birds in Turkey. A total of 125 fish collected in the period between December 2010 and November 2011. Standard parasitological investigation methods were applied and prevalence of infection and mean intensity values were calculated. Morphological diagnostic features of the parasite were studied in detail using light (LM) and scanning electron microscope (SEM). The overall infection prevalence (%) and mean intensity values were determined to be 21.2% and 7.11 ± 3.52 per infected fish, respectively. Infection parameters were also determined in relation with host length, sex and season. Gradual increases of both infection indices in relation with increasing host length sizes, higher indices on male fish (23.7% and 12.07 ± 6.63) and in winter (31.7% and 8.55 ± 4.72). This study presents detailed morphological features of *Clinostomum complanatum* and this is the first record of *C. complanatum* in a new host *Aphanius danfordii.* Results obtained in the present study also make contribution to our current knowledge on this parasite species regarding its host diversity.

DIGENEAN PARASITE DIVERSITY IN TELEOST FISHES FROM THE LOWER KIZILIRMAK DELTA, TURKEY

T. ÖZTÜRK[1*], A. ÖZER[1], A. ÇAM[1], D. YıLMAZ KıRCA[1]

[1]*Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000, Sinop, Turkey*

This comprehensive research study was conducted to determine the digenean parasite diversity in sixteen teleost fish species belonging to Mugilidae (*Mugil cephalus, Liza aurata*), Cyprinidae (*Cyprinus carpio, Vimba vimba, Scardinius erytropthalmus, Carasius gibelio*), Gobiidae (*Neogobius fluviatilis, Pomatoschistus marmoratus, Proterorhinus marmoratus*), Cyprinodontidae (*Aphanius danfordii*), Percidae (*Sander lucioperca*), Gasterosteidae (*Gasterosteus aculeatus*), Poeciliidae (*Gambusia affinis*), Atherinidae (*Atherina boyeri*), Cobitidae (*Cobitis taenia)* and Syngnathidae (*Syngnathus acus*) in the Lower Kızılırmak Delta located by the Black Sea in Turkey. A total of 1049 fish specimens were collected monthly in the period between December 2010 and November 2011. Thirteen digenean species belonging to Heterophyidae (*Ascocotyle felippei*, *A. longa*), Echinostomatidae (*Petasiger* sp., *Echinostoma* sp.), Strigeidae (*Apatemon* sp., *Tetracotyle* sp.), Diplostomidae (*Diplostomum spathaceum, Posthodiplostomum* sp., *Tylodelphys clavata*, *Bolbophorus* sp.), Clinostomidae (*Clinostomum complatanum*), Gorgoderidae (*Phyllodistomum* sp.) and Haplosplanchnidae (*Haplosplanchnus pachysomus*) were identified. Infection prevalence, mean intensity and mean abundance values were calculated for each digenean species on their respective hosts. Parasites were determined to be either host specific or generalist and of the 13 digenean species, 4 (30.8%) infected only single host whereas 2 (15.4%) infected thirteen. *Tylodelphys clavata* and *Posthodiplostomum* sp., found to be dominant species and the previous species had its highest prevalence (78.1%) in *Sander lucioperca* while the latter species had its highest prevalence (89.6%) in *Aphanius danfordii.* The Lower Kızılırmak Delta is protected by the law to be a natural conservation area which has a biological significance for wild life diversity including migratory birds and, considering the complex life cycle of digenean parasites, the results obtained on digenean diversity from sixteen fish species are discussed in detail.

PROLIFERATIVE KIDNEY DISEASE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN CONDITIONS OF INTENSIVE BREEDING: PATHOGENESIS, HEMATOLOGICAL AND IMMUNE PARAMETERS

M. PALÍKOVÁ[1]*, I. PAPEŽÍKOVÁ[1], Z. MARKOVÁ[1], S. NAVRÁTIL[1], L. VOJTEK[2], P. HYRŠL[2], H. SCHMIDT-POSTHAUS[3]

[1]*University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic*
[2]*Masaryk Uuniversity Brno, Brno, Czech Republic*
[3]*Centre for Fish and Wildlife Health, University of Bern, Bern, Switzerland*

In September 2014, we noticed a high mortality of rainbow trout in an intensive Danish type fish culture. The diseased fish exerted pathological changes typical for PKD. Histologically presence of *T. bryosalmonae* was confirmed. Samples of diseased and control fish (healthy fish from another basin) were taken in monthly intervals and haematological, patho-anatomical, histological and immunohistochemical examinations were performed. Water temperature was 14°C in September, 12.5°C in October, 8°C in November, 4°C in December and 3°C in January.
In September, we observed 100% morbidity. Histologically and immunohistochemically we detected ten to hundreds of parasites per high-power field (magnification 400x). Haematological examination revealed decreased numbers of red and white blood cells, low haematocrit values and low concentrations of haemoglobin in diseased fish. On the other hand, complement value, total plasma imunoglobulins, and mainly oxidative burst of phagocytes were significantly elevated.
With decreasing water temperature the mortality stopped. The fish examined one month later showed a better health status and only sporadic or focal presence of parasites; also haematological parameters returned to physiological values. In November only sporadic occurrence of parasites, mainly in kidney tubules, were detected immunohistochemically. Most of the haematological and immune parameters recovered in November or December, respectively. It is evident that despite the poor health state and severe disturbances of the blood parameters, the fish restored organ structure, eliminated the parasites and recovered. The most important factor, along with lowering water temperature, was the activation of fish immune system, especially phagocyte activity.

The study was supported by IGA VFU Brno (246/2015/FVHE) and by project NAZV (QJ 1210013).

THE USE OF A DOUBLE GILL SCORING SYSTEM FOR FIELD EVALUATION OF GILL HEALTH IN ATLANTIC SALMON (*SALMO SALAR* L.)

D. PERSSON, S. KOLSTØ, T.M. PEDERSEN*

FoMAS – Fish health service, Haugesund, Norway

Amoebic gill disease (AGD) on salmon is caused by the protozoan ectoparasite *Paramoeba perurans* witch colonize the gills and causes acute and chronic damage to gill tissue. Typical observations in the acute state are the formation of white mucoid spots and plaques on gill surfaces. Cronical changes are more diverse, but includes necrosis of gill tissue, tissue swelling and a general whitening of single gill lamellas.

An optimal treatment strategy are often based on the development in gross gill score, thus is a score system focusing on the acute and treatable damages an advantage. On the other hand, the severity of the chronic damages and pathological changes caused by other pathogens, provide vital information regarding gill function and fish health status. All which needs to be considered to maintain animal welfare during treatment. It is our experience, from several years of working with AGD along the south-west coast of Norway that we need a divided gross gill scoring system that take in to account both acute and chronical damages on gill tissue. We present a modified gross gill score system with two scores called “active gill score” and “total gill score”.

The active gill score is similar to the score system described by Taylor et al (2009), whereas visible white patches are described on a scale from clear to heavy (score 0-5). We define the active gill score to include all white mucoid spots and plaques typical for the acute phase of amoebic gill disease. The total gill score includes all visible pathology on the gills, both acute and chronic damages caused by AGD and other pathogens or irritants. The observed changes are described on the same scale from clear to heavy (score 0-5) as for the active gill score system. When using this combined scoring system, all gill arches are observed, however only the most affected arch determine the score of the individual fish.

When using total and active gill score continuously on salmon farms during an AGD outbreak they have shown to be an effective and successful tool in handling this disease.

OCCURRENCE AND EFFECTS OF *NEOMETANEMATOBOTHRIOIDES* SP., A DIDYMOZOID PARASITE OF WILD GREATER AMBERJACK IN THE WESTERN MEDITERRANEAN SEA

A. REPULLÉS-ALBELDA[1]*, J.A. RAGA[1], F.E. MONTERO

[1]*Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain*

Neometanematobothrioides periorbitalis is a trematode parasite species of the family Didymozoidae which was first described in greater amberjack (*Seriola dumerili*) from Hawaii. According to the original description, *N. periorbitalis* is a large trematode which reaches up to more than 8cm long and is found "*freely, though entangled in periorbital adipose tissue*" of greater amberjack but its effects have not been studied yet. As a result of a preliminary study on the parasite fauna of Mediterranean greater amberjacks, several specimens of *Neometanematobothrioides* sp. were found. The aim of this work is to describe the seasonal occurrence and effects of *Neometanematobothrioides* sp. infecting wild greater amberjacks in the western Mediterranean. To achieve this objective 185 greater amberjacks from Majorca were periodically sampled; parasites were counted and collected, also recording the infection-site. Moreover, different samples were preserved in formaline 10% to perform histological analyses. Prevalence ranged from 33 to 100% while intensity ranged from 1.7 to 3.9 parasites per fish and no seasonal pattern was detected. Interestingly, specimens were mostly found in gills, along the arches at gill filament bases; partially free inside the visceral cavity or on the peritoneum. No worms were found in periorbital tissues. Infected fish did not show apparent pathologies or effect on fish condition and no specific reaction was detected in tissues surrounding the parasites. *Neometanematobothrioides* sp. has been also reported in cultured *S. dumerili* from Murcia (Spain) with no apparent pathological effect. In this case parasites were also found in the periorbital tissues. Therefore, these parasites would be considered harmless for greater amberjack, except for some devaluation of the aspect due to the apparent yellowish tracts on fish viscera.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

IN VITRO ANTIMICROSPORIDIAL ACTIVITY OF GOLD NANOPARTICLES

M. SALEH[1]*, G. KUMAR[1], A. ABDEL-BAKI[2], S. AL-QURAISHY[2], M. EL-MATBOULI[1]

[1]*Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria*
[2]*Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia*

The microsporidian parasite *Heterosporis saurida* affects lizard fish, *Saurida undosquamis* causing severe economic losses in marine aquaculture. Only a few drugs are permitted for the treatment of parasitic infections in aquaculture. To overcome this problem, new materials are being tested. Recently, more interest has been directed towards metal nanoparticles which exhibit novel chemical and physical characteristics owing to their extremely small size and high surface area to volume ratio. The present work has been made an attempt to investigate the antimcrosporidial efficacy of gold nanoparticles to hamper the growth of the fish microsporidian *H. saurida*. Gold nanoparticles were synthesized by the chemical reduction method of tetrachloroauric acid as a metal precursor. The antimicrosporidial efficacy of gold nanoparticles against *H. saurida* was assessed using an in vitro screening approach. The number of *H. saurida* spores in infected eel kidney cell line (EK-1) was reduced in a proportional manner to the concentrations of gold nanoparticles tested. When the MTT test was carried out, gold nanoparticles did not appear to be toxic to the host cells. From this study, it is concluded that gold nanoparticles could act as an alternative choice for development of new antimicrosporidial drugs to combat disease problems in aquaculture.

SERIOLA DUMERILI PARASITISED BY THE SKIN MONOGENEAN *NEOBENEDENIA MELLENI* ON SPANISH ATLANTIC CULTURES

N. SÁNCHEZ-GARCÍA[1], A. REPULLÉS-ALBELDA[1]*, J.Z. COSTA[2], J.A. RAGA[1], F.E. MONTERO[1]

[1]*Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain*
[2]*Moredun Research institute, Penicuik, UK*

This study shows outbreaks of *Neobenedia melleni* on *Seriola dumerili* cultured in Canary Islands. Monogenan capsalids of the genus *Neobenedenia* are common skin and gill parasites of marine fishes, frequently highly pathogenic. The species of this genus usually show low host specificity, what hampers their control and epidemiological follow-up. *Neobenedenia* spp. parasitations of some commercially important fish species, as *Seriola dumerili* and *S. quinquerata*, have been reported to cause high mortalities and considerable economic losses in marine aquaculture facilities in the western Pacific (Japan and Australia).
The goal of present study is to identify the species of *Neobenedenia* sp. parasitising the cultures of *S. dumerili* in Canary Islands (Eastern Atlantic). The identification of the species of *Neobenedenia* was made through their morphological study and confirmed with molecular analyses due to the controversial taxonomy of the group. To complete the identification, the isolated specimens were compared with morphologic and morphometric descriptions and available sequences of *N. melleni* and *N. girellae.*
We conclude that the monogeneans herein analysed correspond to the species *N. melleni* which has not been reported to date in wild or cultured fish from the Atlantic waters. The presence of *N. melleni* can cause health fish problems and economic losses in the Atlantic cultures of *S. dumerili* what points out the importance of developing a proper monitoring and management planning in the aquaculture installations.

Funded by ISIC/2012/003 & PROMETEO 795/2015/018 projects.

INVESTIGATION OF PARASITES IN SOME FİSH CULTURED IN VI. REGIONAL DIRECTORATE OF STATE HYDRAULIC WORKS, FISHERIES HEAD ENGINEERING

S. TABAKOGLU, İ.CENGİZLER, M. ETYEMEZ

Department of Aquaculture and Fish Diseases, Faculty of Fisheries, University of Cukurova, Adana 01330, Turkey

Ecto-parasites on the skin, fins, gills and intesitines of *Cyprinus carpio* (mirror carp), *Cyprinus carpio* (common carp) and *Ctenophoringodon idella* (grass carp) from *Cyprinidae* family in the fresh water aquaculture station of Vı th Regional Directorate of State Hydraulic Works were investigated and distributions according to months were made in this study. The study was carried out between September 2002 and August 2003 and 360 individuals in total were used. Although it could not be found any endo-parasites, *Lernaea cyprinacea* (Linnaeus, 1746), *Argulus foliaceus* (Müller, 1785), *Ichthyophthirius multifiliis* (Fouquet, 1876), *Dactylogyrus vastator* (Nybelin, 1924), *Chilodonella cyprini* (Moroff, 1902) and *Trichodina perforate* (Lom, 1961) were recorded at the end of the study. The rate of ecto-parasites was high in autumn and winter and low in spring and summer.

EFFECTS AND IMPLICATIONS IN GILTHEAD SEABREAM (*SPARUS AURATA*) OF GILL AREA AFFECTED BY *SPARICOTYLE CHRYSOPHRII*

M. VILLAR-TORRES, J.A. RAGA, F.E. MONTERO, A. REPULLÉS-ALBELDA*

[1]*Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain*

Sparicotyle chrysophrii is one of the most harmful metazoan parasites infecting wild and cultured gilthead seabream (*Sparus aurata*) in the Mediterranean. This is a polyopistocotylean monogenean species whose pathological effects are associated to its attachment and feeding injuries. *Sparicotyle chrysophrii* specimens attach to its host by a posterior haptor that allow them to remain on the gills. Apart from its mechanical action, this haptor covers a portion of gill surface, diminishing the breathing interface and causing thus a pathological effect. This study deals with the analysis of the significance of the covered gill area in hosts with different sizes. To achieve this objective, two hundred and sixty-nine adult specimens were removed from one hundred and forty-five gilthead sea breams of different sizes, stained, mounted on permanent slides and measured, obtaining different dimensional variables. Fish gills were also measured in fresh and after treatment with histological methods. As a result of this study, similar growth rates were detected for parasites infecting gilthead seabreams of different sizes. Moreover, parasite specimens, as well as their haptors, were significantly larger in longer fish. In the same way, wider clamps of the haptor were also significantly wider in specimens infecting longer hosts. According to these findings, the area of the gills covered by the haptor of monogeneans would be proportionally similar for fishes differently sized. Therefore, unless parasite load was different, pathological effects linked to attachment should be similar. In the present study no significant relationship was found between number of parasites and host size. However, this fact should be analysed and considered, together with parasite-host sizes and growth, for damage assessments.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

PARAMOEBA PERURANS – MORPHOLOGY AND GILL INTERACTIONS IN ATLANTIC SALMON (*SALMO SALAR* L)

J. WIIK-NIELSEN[1]*, M. GJESSING[1], H. KOLSTAD[2], S.N. MOHAMMAD[1], S. HYTTERØD[1], M.D. POWELL[3]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*Norwegian University of Life Sciences, Imaging Centre, Ås, Norway*
[3]*Norwegian Institute for Water Research, Bergen, Norway*

Amoebic gill disease (AGD) caused by the amphizoic amoeba *Paramoeba perurans* has recently become a disease of significance to Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum) aquaculture in Northern Europe.
Few studies have investigated amoebic gill disease at the ultrastructural level. In the present study, the morphologies of cultured *Paramoeba perurans* and the surface relationships between *P. perurans* with the Atlantic salmon gill epithelium in the development of AGD will be presented.

MOST ABUNDANT PROTEINS FROM SEMINAL PLASMA MAY AID PROTECTION OF THE TESTIS VIA ANTIMICROBIAL ACTIVITY.

M. ADAMEK[1]*, M. DIETRICH[2], J. NYNCA[2], A. CIERESZKO[2], D. STEINHAGEN[1]

[1] *Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2] *Department of Gamete and Embryo Biology, Polish Academy of Sciences, Olsztyn, Poland*

Testis is known to be an immune-privileged organ with a blood-testis barrier. It is capable of mounting effective immune responses, but with a constitutive expression of anti-inflammatory cytokines it is considered to be locally immunosuppressed. Maintenance of homeostasis in the gonadal tissue is essential for successful gametogenesis and fertilisation. For this, multifunctional proteins with antimicrobial activity may play a significant role and can also provide protection to the organ. Proteomic analyses of common carp and rainbow trout seminal plasma indicated several molecules as candidates for such multifunctional proteins, including transferrin, serine proteinase inhibitors, apolipoproteins and warm temperature acclimation protein Wap65, which were all present in high abundance and were shown to have an antimicrobial function in blood plasma.
The aim of the presented study was an evaluation whether selected proteins from the seminal plasma are expressed locally in the testis of common carp and rainbow trout. This was done by RT-qPCR based analysis of mRNA expression. Furthermore the antibacterial and antiviral activity of these proteins was estimated by a co-incubation of several bacteria (*Escherichia coli* JM109, *Aeromonas hydrophila* BSK10 and *Pseudomonas fluorescens* W284) and viruses (CyHV-3, VHSV) with selected proteins isolated from the seminal plasma. The activity was evaluated by bacteria/virus quantification using colony forming unit (CFU) plating assays or 50% Tissue Culture Infective Dose ($TCID_{50}$) assays.
The results indicated that although the genes encoding for all the proteins in question were expressed in testis, their level of expression was different from the level recorded in liver. For example the genes encoding for apolipoproteins and transferrin were expressed significantly higher in liver, while the gene encoding for the serine protease inhibitor Kazal-type 2 was significantly higher expressed in testis. Most of the proteins showed at least a bacteriostatic activity and apolipoproteins had a very strong bactericidal activity, especially against *E. coli*.
Based on these results, the testis seems to have the capacity to produce the most abundant proteins in seminal plasma. These proteins have an antimicrobial activity and could considerably contribute to the protection of this organ, especially from bacterial infections.

This work was supported by NSC-Poland Projects 2011/01/D/NZ9/00628, 2011/01/D/NZ9/00619.

THE *SENEGALESE SOLE Mx* GENE PROMOTER CONTAINS A VARIABLE MICROSATELLITE REGION INVOLVED IN THE TRANSCRIPTIONAL CONTROL

D. ALVAREZ-TORRES*, L.P. VIEYTES, E. GARCIA-ROSADO, M.C. ALONSO, J. BEJAR

Universidad de Málaga, Málaga, Spain

Interferons (IFNs) play a key role in the fish innate immune system against viral infections by stimulating the expression of IFN stimulated genes (ISGs), such as *Mx*. The mechanisms involved in the transcriptional regulation of fish ISGs are poorly understood. The sequencing of the Senegalese sole (*Solea senegalensis*) *Mx* gene (*SsMx*) promoter revealed the presence of a guanosine-citosine (G-C) rich region that contains a microsatellite, sited close to the translation start codon. The aim of the current study has been to determine the functional role of this region and to study its variability.

In order to fulfill the first objective, RTG-2 cells were transiently transfected with a vector containing the luciferase reporter gene under the control of the wild type *SsMx* promoter or the mutated promoter in which the G-C region was removed. Afterwards, transfected cells were treated with poly I:C and the luciferase activity was measured at different times post-stimulation. The results indicated that this region is involved in the *SsMx* transcriptional control, being an essential motif for the *SsMx* promoter activity.

In addition, the microsatellite variability was studied in different sole populations, in which four different alleles have been found. The differences in the genetic structure of this locus among these populations have been analyzed. Microsatellites in gene promoters are commonly involved in the transcriptional regulation, and allelic differences are frequently related to the promoter activation level. For this reason, it is tempting to suggest that the polymorphisms found in the G-C microsatellite of the *SsMx* promoter, which has not been previously described in any teleost *Mx* gene promoter, might have a functional meaning. However, further studies are required in order to establish a functional relationship between the different alleles and the level of *SsMx* transcription. In this approach, it would be mostly interesting to evaluate if this variability is related to the Senegalese sole resistance to viral infections. If such relationship exists, this locus could be used as a marker for genetic selection of this fish species.

This study has been funded by the P09-CVI-4579 project, from Junta de Andalucía (Proyectos de Excelencia de la Junta de Andalucía).

ANTIMICROBIAL ACTIVITY AND *IN VIVO* PROTECTION OF SYNTHETIC HEPCIDIN AGAINST *VIBRIO ANGUILLARUM* IN SEA BASS (*DICENTRARCHUS LABRAX*)

C. ÁLVAREZ[1, 2], F. ACOSTA[3], B. VEGA[3], D. MONTERO[4], F. GUZMÁN[5], P. SANTANA[1], E. TORRES[1], L. MERCADO[1]*

[1]*Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso*
[2]*Doctorado en Biotecnología, Universidad Técnica Federico Santa María/Pontificia Universidad Católica De Valparaíso, Chile*
[3]*Grupo de Inmunología Veterinaria y Producción Animal, Universidad de Las Palmas de Gran Canaria, Arucas, España*
[4]*Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria, España*
[5]*Nucleo Biotecnología Curauma, Pontificia Universidad Católica De Valparaíso, Chile*

The generation of a variety of new therapeutic agents to control and reduce the effects of pathogen in aquaculture is urgently needed. *Vibrio anguillarum* is a global marine pathogen able to infect a wide spectrum of farmed fish species at various salinity and temperature conditions. In addition, *V. anguilarum* antimicrobial resistances in different fish species have been reported. Antimicrobial molecules are important members of the host defense peptides system in low and high vertebrates. These peptides are potent, broad-spectrum antibiotics that demonstrate potential as novel and alternative for the treatment of drug resistant infections. Therefore, we investigated the ability of different variants of hepcidin antimicrobial peptide to protect against infection caused by *Vibrio anguillarum*. The peptides were synthesized by a solid-phase method using the Fmoc procedure and the molecular mass and purity were confirmed by ESI-MS and HPLC respectively. To identify effective synthetic variant, antibacterial activity was determined using the microplate assay. All peptides were able to strongly inhibit the growth of Vibrio at 100μM and one variant showed best inhibition at lowest concentration. Thus, this peptide was selected for in vivo studies. To analyze the protection of synthetic peptide, European Seabass were intraperitoneally injected with 25 μg of hepcidin variant or phosphate buffer and after two hour post injection; the fish received intraperitoneal injections of *V. anguillarum* at a lethal dose 50. The synthetic peptide did not show cytotoxic effects and significantly reduced the accumulated mortalities percentage (25%) than the European Seabass injected with phosphate buffer previously (80%) at day 10. Vibriosis mortality was confirmed by isolating of *V. anguillarum* from liver and spleen of dead fish. In conclusion synthetic hepcidin variants show antimicrobial activity against *V. anguilarum* and is proposed as a possible new agent for the protection of vibriosis infections in aquaculture.

PIECING TOGETHER THE PROLIFERATIVE KIDNEY DISEASE PUZZLE: DOES TEMPERATURE MODULATE THE IMMUNE RESPONSE?

C. BAILEY, H. SEGNER, T. WAHLI

Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Switzerland

Proliferative kidney disease (PKD) is a chronic disease of freshwater salmonids caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. This parasite is a common pathogen in aquaculture and watercourses of Europe and North America, predominantly in low land rivers suggesting an influence of water temperature on infection dynamics.
Gene expression data indicate that *T. bryosalmonae* causes a chronic lymphoid immunopathology in the kidney of infected fish. To confirm or reject this hypothesis, a further exploration of the cellular immune response is required. So far such a study has been thwarted by the absence of appropriate antibodies.
Such antibodies for FACS or immunohistochemical studies of fish have not yet been extensively applied. In the present study, by using newly available antibodies we were able to discriminate between major leukocytes subpopulations and to recognize specific B cell subsets, in both the anterior and posterior kidney of fish. This was reinforced by the measurement of transcription factors that were differentially expressed in immune relevant genes and during stages of B cell development, resulting in elucidating some of the mechanisms promoting the cellular immune response in PKD affected rainbow trout. Fish were kept at two different temperatures and exposed to the same quantity of *T. bryosalmonae* spores, to determine whether the fish immune response and/or parasite intensity is influenced by water temperature, and how these parameters interact. Results demonstrated a distinct influence of water temperature.

HISTOPATHOLOGICAL FINDINGS IN *AEROMONAS SALMONICIDA SUBSP. SALMONICIDA*-INFECTED TURBOT (*SCOPHTHALMUS MAXIMUS*). WHY THE TERM "FURUNCULOSIS" SHOULD BE AVOIDED?

R. BERMÚDEZ[1]*, G. COSCELLI[2], A.P. LOSADA[1], P. RONZA[1], Y. SANTOS[3], M.I. QUIROGA[1]

[1]*Facultad de Veterinaria*
[2]*Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Argentina*
[3]*Facultad de Biología (CIBUS), Universidad de Santiago de Compostela, Spain*

Aeromonas salmonicida subsp. *salmonicida* (*A. salmonicida*), the etiologic agent of "classical furunculosis", is one of the most important fish pathogen in aquaculture worldwide, which mainly affects both farmed and wild salmonid species. In salmonids, different clinical presentations of the disease have been described, varying from hyperacute and acute forms, to chronic or even asymptomatic infection. The acute form is the most common presentation of the disease in both juvenile and adult fish, resulting in a hemorrhagic septicaemia with high mortality rates. Chronic lesions associated to the diseases include erosions, ulcers and boil-like lesions named "furuncles". *A. salmonicida* has also been isolated from a wide variety of commercially valuable non-salmonids species, including turbot, halibut, cod and Senegalese sole. Turbot aquaculture represents an important economic activity in several countries of Europe, Asia and South America, and epizootic outbreaks of acute furunculosis in turbot farms have been reported in several countries. While *A. salmonicida* infection in turbot generally results in an acute fatal disease, chronically infected specimens show cutaneous nodules and ulcers on the ocular surface. Diagnosis of this disease in fish is commonly made by pathogen isolation, serological or molecular techniques. However, microscopic and immunohistochemical evaluations of lesions are not routinely performed and detailed information about pathogenesis and pathological findings in turbot is very limited. In this study, turbot coming from natural outbreaks of the disease or obtained from experimental infections were employed in order to achieve a complete morphopathological description of the disease in this species. Moreover, an immunohistochemical technique was developed to detect *A. salmonicida* antigen in host tissues, in order to study the pathogenesis of the disease. Acute form of infection by *A. salmonicida* in turbot was characterized by septicaemia, generalized vascular changes and presence of the bacteria in target organs. In the chronic form, a moderate multifocal granulomatous dermatits was noted, which demonstrated the involvement of the cellular immunity and the development of cell-mediated hypersensibility. The use of the terms "furuncle" for the description of the lesions in turbot and "furunculosis" will be discussed.

AN *IN VITRO* STUDY OF COMMON CARP (*CYPRINUS CARPIO*) ANTIGEN PRESENTING CELL FUNCTION

I. CANO[1], E. GUZMAN[2], R. PALEY[1], R. VAN AERLE[1]

[1]*Cefas, Weymouth Laboratory, Weymouth, UK*
[2]*The Institute for Animal Health, Pirbright, UK*

Antigen presenting cells (APC) are central in the development and maintenance of immune responses. Of these, dendritic cells (DC) have been shown to be the most potent APC subset.
Recently, a teleost DC model has been described (Bassity & Clark, 2012), whereby cells are harvested from head kidney and cultured *in vitro*. This results in the maturation of phagocytic mononuclear cells with DC-like morphology.
A non-adherent cell population derived from common carp (*Cyprinus carpio*) monocytes showing branched morphology was analysed to study the expression of DC markers. A number of markers were found to be transcribed in DC, including TLR20, TLR22, CXCR4, CXCR7, MHCII and IL1ß. In contrast, TLR9, TLR10 and IL12 were only found to be transcribed in carp head kidney samples.
Two CD83 putative genes, CD83 and CD83-like, were identified in the carp genome and their deduced amino acid sequences shared 56% and 25% homology respectively with rainbow trout CD83. Both CD83 putative genes were transcribed in the DC population, although CD83-like expression was 4 times higher than CD83.
In order to study activation of DC by pathogen associated molecular patterns (PAMS), DCs were exposed to heat inactivated cyprinid herpesvirus 3 (CyHV3). Confirmation of DC activation was determined by TaqMan qPCR analysis of various markers, including CD83 and MHCII. After 1 day of exposure with non-replicative CyHV3 both carp CD83 putative genes were up-regulated in the five replicates analysed, with CD83 showing a higher fold increase in gene expression than the CD83-like gene. However, MHCII did not show a significant fold increase after one day of DC activation with viral antigen. In ex *vivo* samples, CD83-like was only found to be transcribed in 2 out of 7 of the CyHV-positive head kidney samples, and CD83 just in 1 out of 7. Neither CD83 genes were found to be transcribed in CyHV-negative head kidney samples.
Understanding APC function in fish underpins the development of vaccines for current and emerging infectious diseases. *Ex vivo* culture of DCs could be an appropriate *in vitro* model to study teleost APC function.

DNA VACCINATION OF BROODSTOCK SPECIMENS MODIFIES THE IMMUNE STATUS OF THEIR PROGENY

Y. VALERO[1], A. CUESTA[2], A. GARCÍA-ALCÁZAR[1], M.A. ESTEBAN[2], E. CHAVES-POZO[1]*

[1]*Instituto Español de Oceanografía, Puerto de Mazarrón, Spain*
[2]*University of Murcia, Murcia, Spain*

Transfer of maternal immunity is a term attributed to the immune factors transferred from mother to offspring in order to protect the progeny, while their own immune response is still undeveloped. This kind of transfer has been described in vertebrates, including fish. In teleost fish, it has been reported that a maternally-derived immunoglobulin (IgM) is contained in the oocytes, eggs and/or larvae together with other non-specific immune factors such as complement factors, lectins and lysozyme. This transferred immunity gradually declines with time, however, the control of this process could lead to develop specific maternal vaccination against eggs and larvae pathogens and vertically transmitted virus. Nodavirus (VNNV), a demonstrated vertical transmitted virus, causes the viral encephalopathy and retinopathy (VER) disease, particularly in European sea bass larvae and juveniles, provoking high mortality rates. Although viral diseases are responsible for many economic losses in modern aquaculture producing high mortalities, they have no effective antiviral treatments available yet. In this study we intramuscularly injected an experimental DNA vaccine against VNNV to five female broodstock specimens and studied the improvement of the immune system status at functional and gene expression levels in the eggs and larvae of their progeny with the aim of determining whether the vaccine can provide a higher status of immunity to the offspring. Our data determined some improvement, however further characterization of the mechanism involved in the transfer of maternal immunity in fish are needed.

Financial support by grants AGL2010-20801-C02-01, AGL2010-20801-C02-02 and AGL2013-43588-P and RYC-2009-05441 (MINECO and FEDER) and grant to Y. Valero (Instituto Español de Oceanografía) is gratefully acknowledged.

VACCINE EFFICACY OF GLYCERALDEHYDES-3-PHOSPHATE DEHYDROGENASE AND COPPER BINDING PROTEIN FROM *NOCARDIA SERIOLAE* AGAINST NOCARDIOSIS IN LARGEMOUTH BASS

S.C. CHEN[1]*, H.E. HUNG[1], H.H. HU1, M.A. TSAI[1], P.C. WANG[1], L.L. LIAW[2]

[1]*College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan, Republic of China*
[2]*Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, Republic of China*

Nocardiosis caused by *Nocardia seriolae* is an important disease in aquaculture industry worldwide. Characteristics of *N. seriolae* are gram-positive, acid-fast bacilli, non-motility, which have abscesses in the epidermis and characterized by systemic nodulation, manifested both on the skin and internal organs in fish. Recently, there were more and more fishes being infected such as largemouth bass (*Micropterus salmoides*), grey mullet (*Mugil cephalus* Linnaeus), three-striped tigerfish (*Terapon jarbua* Forsskal) and spotted butterfish (*Scatophagus argus*, Linn) and resulted in a mass mortality in Taiwan. However, until recently no vaccine has been available for prevention of infection with this organism. In this study, copper binding protein (rCBP) and glyceraldehyde-3-phosphate dehydrogenase (rGAPDH) genes from *N. seriolae* have cloned and expressed for development of subunit vaccines in largemouth bass. Western blotting assay revealed that rCBP and rGAPDH shared the common antigenicity with *N. seriolae* infected sera from largemouth bass and it reacted with antigen at 18.8 kDa and 43.2 kDa, respectively. Further experiment, largemouth bass were immunized intraperitoneally with rCBP and rGAPDH (100 μg $fish^{-1}$), respectively. ISA 763A was used as an adjuvant for vaccine and phosphate-buffered saline (PBS) was used as a negative control. The fish challenged at 4 weeks after immunization with rGAPDH+ ISA had the relative percent survival (RPS) at 62.5%, followed by fish immunized with rCBP+ISA, which had RPS values of 50%. Our results further demonstrated that rCBP and rGAPDH from *N. seriolae* protected largemouth bass from experimental *N. seriolae* infection, implying the potential use of *N. seriolae* rCBP and rGAPDH as subunit vaccines against *N. seriolae*.

INDOLEAMINE 2,3-DIOXYGENASE (IDO) AS A POTENTIAL INDICATOR OF THE IMMUNE RESPONSE AGAINST PATHOGENS IN RAINBOWN TROUT

L.J. CORTÉS[1], C. ÁLVAREZ[1], E. TORRES[1], F. GÓMEZ[1], L. MERCADO[1, 2]*

[1]*Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología. Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[2]*Núcleo Biotecnológico de Curauma (NBC). Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*

Fish aquaculture has grown rapidly worldwide in the last years. This phenomenon has involved the development of molecular markers as physiological and animal health indicators. Natural resistance to pathogens is one of the main features wanted in cultured fish. In this regard immune system is the target for searching molecular markers of robustness in animal health. Enzymes have been suggested as active actors against pathogens and considered excellent molecular markers of innate immunity in teleost fish. Indoleamine-2,3-dioxygenase (IDO) is an enzyme that catalyzes degradation of L–Tryptophan (L-Trp) in local tissue microenvironments, has been described in higher vertebrates. This ability of IDO to deprive cells of L-Trp and preventing the normal growth of pathogens shows its biological roles of host innate immune defense and immune control. In the present work a full-length transcript encoding for IDO was isolated from rainbow trout *Oncorhyncus mykiss* –*Om*IDO- and functionally characterized. Coding sequence was amplified and cloned using primers design from whole genome rainbow trout sequence database. Sequencing confirmed that the isolated gene encoded a protein that belongs to IDO-like-protein described in *Danino rerio*. Additionally, phylogenetic analysis classified this protein as a member of the IDO2-family, the probable ancestor of the IDO1 present in higher vertebrates, protein specialized in tryptophan degradation. This gene is constitutively expressed in different fish organs, moreover when animals were challenged with LPS, the IDO-expression increase in different mucosal systems like gills, skin and gut. IDO-expression induced by type II interferon in response to Pathogen-Associate Molecular Patters (PAMPS) has been described previosly in higher vertebrates. We demonstrated the in vitro induction of *Om*IDO by rIFN-γ using a rainbow trout cell line derived from fish spleen, RTS-11. These findings allow proposing this protein as molecular marker of robustness of the immune system in order to evaluate the animal response to immunomodulators, different diets and genetic improvement in the phenotypic characterization of cultured rainbow trout.

ORAL ADMINISTRATION OF A NODAVIRUS DNA VACCINE INTO CHITOSAN NANOPARTICULES IMPROVES THE SURVIVAL OF EUROPEAN SEA BASS JUVENILES UPON CHALLENGE

Y. VALERO[1], E. AWAD[2], E. CHAVES-POZO[1], J. MESEGUER[3], M.A. ESTEBAN[3], A. CUESTA[3]*

[1]*Instituto Español de Oceanografía, Puerto de Mazarrón, Spain*
[2]*National Research Center, Giza, Egypt*
[3]*University of Murcia, Murcia, Spain*

Vaccines are considered one of the most effective methods for the improvement of the immunity in fish against a particular disease or a group of diseases. Nodavirus (VNNV) causes the viral encephalopathy and retinopathy (VER) disease, producing many economic losses in the modern aquaculture because of the high mortality rates provoked, especially in juveniles and larvae. Different vaccine administration routes such as injection, immersion or oral vaccination show variable effects. Thus, oral vaccination is the most preferred method to be used in aquaculture because it is no invasive, cheap and ideal for mass administration of fish of all size without handling stress. Several vaccines have been developed against nodavirus including; inactivated, recombinant and DNA vaccines. On the other hand, chitosan is a polysaccharide extracted from crustacean shells widely used as a polycationic gene carrier. In this study we orally vaccinated healthy specimens of European sea bass with a body weight of 6 g, one of the most sensitive species to VNNV, with a DNA vaccine adhered to chitosan nanoparticles and included in the commercial diet of the specimens and evaluated the immune response and the survival rates after a challenge with VNNV. Normal commercial diet and diet with chitosan nanoparticle and the empty plasmid adhered to chitosan nanoparticles were used as controls. Our results show that this oral vaccine increases the survival rates and stimulates the immune response by up-regulating *ifn*, *mx*, *tcrb* and *cd8* gene expression. Otherwise, empty plasmid and chitosan showed similar profiles but with significantly lower results. These results point to the improvement of the anti-viral mechanism to fight against of VNNV.

Financial support by grants AGL2013-43588-P and RYC-2009-05441 (MINECO and FEDER), 04538/GERM/06 (Fundación Séneca, Spain) and grant to Y. Valero (Instituto Español de Oceanografía) is gratefully acknowledged.

NOD-LIKE RECEPTOR AND TRIM PROTEINS AS IMMUNOLOGICAL INDICATORS OF INNATE IMMUNE RESPONSE IN GILL OF *ONCORHYNCHUS MYKISS*

F. DONOSO[1], F. RAMÍREZ[1], C. ALVAREZ[1], P. SANTANA[1], F. GUZMÁN[2], L. MERCADO[1, 2]*

[1]*Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología. Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[2]*Núcleo Biotecnológico de Curauma (NBC). Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*

The intensity of the salmon aquaculture industry has resulted in the rise and persistence of different viral and bacterial pathogens. One weakness of salmonid culturing is the lack of knowledge about the immune response in fish under culturing conditions, which includes the mechanism of the pathogen recognition. Innate immune responses are triggered by the stimulation of pattern recognition receptors (PRRs) by pathogen-associated molecular patterns. The activation of PRRs initiates specific immune responses, such as the cytokines and immune effectors secretion. One of them are interferons (IFNs) produced in response to these stimuli and are crucial for the induction of effective immunity. One type of PRRs is the NOD-like receptors (NLR) family which, in higher vertebrates, induces the expression of the cytosolic superfamily of tripartite motif-containing (TRIM) proteins. These are ancestral molecules present in biological systems before cytokines and with the capacity for differential expressions in response to viral or bacterial PAMPs. In fish, the cellular and molecular mechanisms of the innate immune response are analogous than in higher vertebrates. Our previous research has shown the versatility of the immune response in gill tissue and how this tissue can be a model for evaluating the immunological status of the animal. In order to contribute in this field, different types of transcripts encoding for NLR and TRIM proteins were identified in response to PAMPs in a cell line from gill epithelium of *Oncorhynchus mykiss* (RTgill-W1). The mRNA expression of these receptors and cytosolic transducer was assessed by qPCR using LPS and Poly I:C as bacterial or virus PAMPs, respectively. The results showed that the up-regulation of different NLR or TRIM proteins is dependent of the inducer used. Additionally, the cytokine profile induced was evaluated and the expression of proinflammatory and interferons was established for each type of PAMPs. The results obtained contribute to different strategies of detection and quantification that can be utilized as a diagnostic tool for detecting differentiated viral or bacterial pathogenic agents, thus contributing to the health management of salmonid cultures.

FONDECYT 1140797.

ALTERATIONS IN THE EXPRESSION OF ANTIMICROBIAL PEPTIDE GENES ON THE MUCOSAL SURFACES OF ATLANTIC SALMON

Y. KITANI*, J.M.O. FERNANDES, V. KIRON

Faculty of Biosciences and Aquaculture, University of Nordland, Bodø, Norway

Detection of diseases at an early stage can prevent disease-associated losses in aquaculture. The first host barriers that encounter pathogens are the skin and gills. Information on candidate molecular markers on these mucosal surfaces will be valuable for the fish farming industry. Therefore, in different experiments, we examined the responses of antimicrobial peptide (AMP) and host-defense protein genes – beta-defensins, cathelicidins, hepcidin and NK-lysin – on the skin and gills of Atlantic salmon (*Salmo salar*) by real-time PCR.

The AMP expression on the skin of salmon that were infested with sea lice at day-10 following the challenge was examined. Next, *Aeromonas salmonicida* bacterin IP injection-induced alterations in AMP transcript levels in the skin and gills of the fish at 1 day and 7 weeks (500 day °C) after injection were studied. In an in vitro time-course study, the primary gill tissue cultures were exposed to the bacterial pathogens, *A. salmonicida* and *Moritella viscosa*, and the AMP gene responses were evaluated at 12, 24, 48 and 96 h after exposure. Finally, alterations of the genes in fish subjected to an increase in the temperature from 7 to 18 °C were also assessed.

The results from these experiments suggest that some of the AMPs may well be used as diagnostic markers of the pathogen infection / stress in Atlantic salmon.

EFFECTS OF IMMUNONANOLIPOSOMES IN TERMS OF IMMUNOLOGICAL STIMULATION AND PROTECTION AGAINST YERSINIOSIS IN RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*, WALBAUM)

B. FOUZ[1]*, A. LLORENS[1], A. RUYRA[2], M. CARMONA[2,] N. ROHER[2]

[1]*Universitat de València, Valencia, Spain*
[2]*Universitat Autònoma de Barcelona, Barcelona, Spain*

Vaccination with bacterins has achieved great success for the control of Enteric Redmouth (ERM) disease, caused by *Yersinia ruckeri* (*Yr*), in trout farming. However, from 2001, unmotile isolates of *Yr* have been recovered from episodes of ERM in vaccinated rainbow trout over the world (Fouz et al., 2006). A promising strategy to improve the fish immune response could be to use nanocarriers as delivery vehicles of subcellular vaccines or immunostimulants (Ruyra et al., 2013). The main objective of this study have been to evaluate the effects of the administration of lipid formulations (nanoliposomes, NLs) loaded or not with immunological relevant molecules in terms of both immune system stimulation and protection against yersiniosis in rainbow trout. For this purpose, we performed a controlled laboratory trial in which cultured fish were immunized with empty NLs (controls) or NLs loaded with Poly (I:C) +LPS (NLc) during a four week-period. Subsequently, we challenged fish with *Yr* using an intracoelomic (ic) injection model.

Evaluation of the efficacy of treatments against ERM disease was based on differences in mortality in fish after challenge. Immune response was assayed measuring IgM levels in plasma and expression of relevant genes in spleen and kidney cells. In NLc group, under the assayed conditions, mortality was lower and occurred later (2-3 days) than in the control group. Significant increase of IgM level as well as of expression of genes related with antigen presentation (MHCII, CD83 or CD8) were also detected at day 3 post-challenge in this group,

In conclusion, administration of NLc has yielded an apparent benefit for trout in terms of both protection against yersiniosis and immune response. Thus, NLc could be a good candidate to be administered to trout before stressing periods and, moreover, these nanocarriers could be use as delivery vehicles of novel vaccines.

References:

- Fouz, B., Zarza, C., Amaro C. (2006). First description of non-motile Yersinia ruckeri serovar I strains causing disease in rainbow trout cultured in Spain. Journal of Fish Diseases, 29: 339-346.

- Ruyra, A., Cano-Sarabia, M., Mackenzie, S., Maspoch, D. and Roher, N. 2013. A novel liposome-based nanocarrier loaded with an LPS-dsRNA cocktail for fish innate immune system stimulation. PLOS ONE. 8: 1-13.

CYTOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF LEUCOCYTES ISOLATED FROM BALLAN WRASSE (*LABRUS BERGGYLTA* A.)

G.T. HAUGLAND*, A. RØNNESETH, H.I. WERGELAND

University of Bergen, Bergen, Norway

We have characterized isolated leucocytes from wrasse (*Labrus bergylta* A.) by morphology and cytochemical staining for myeloperoxidase, acid phosphatase, α-naphtyl acetate esterase (non-specific esterase), naphthol AS-D chloroacetate acetate (spesific esterase), alkaline phosphatase and carbohydrates with periodic acid-Schiff. We could differentiate between several subsets of leucocytes; lymphocytes, monocytes/macrophages, neutrophils, eosinophils, basophils and small leucocytes that might be precursor or immature cells. One striking observation was the eosinophils. The neutrophils had rounded, bean shaped or bi-lobed nuclei and resembled neutrophils in Atlantic cod (*Gadus morhua* L.) and lumpsucker (*Cyclopterus lumpus* L.), but were different from the polymorphonucleated neutrophils in Atlantic salmon (*Salmo salar* L.) and humans. Basophils were observed, but they were rare. Further, we have studied the innate immune responses phagocytosis and respiratory burst using flow cytometry. Highest phagocytic activity was observed among monocytes/macrophages and small leucocytes. Several different subtypes had ability to perform an oxygen-dependent degradation of microbes, measured as respiratory burst activity. Knowledge of the basic properties of wrasse's leucocytes and innate immunology can benefit further studies on its adaptive immune responses.

EXPRESSION ANALYSIS OF THE TOLL-LIKE RECEPTORS INVOLVED IN VIRAL RESPONSE OF NATURAL INFECTED SYMPTOMATIC AND ASYMPTOMATIC SALMON WITH IPN VIRUS

D. HAUSSMANN[1, 2]*, F. LAGOS[1, 2], L. SOTO[1], T. CÁRDENAS[1], J. FIGUEROA[1, 2]

[1]*Universidad Austral de Chile, Valdivia, Chile*
[2]*Centro FONDAP, Interdisciplinary Center for Aquaculture Research INCAR, Concepción, Chile*

The Infectious Pancreatic Necrosis (IPN) is a disease that mainly affects Atlantic salmon (*Salmo salar*), causing large economic losses mainly in juvenile state and pre-smolt fish. This disease is caused by IPN virus, characterized for being a double-stranded RNA virus.
On the other hand in innate immune response, main receptors responsible for recognizing pathogens, such as virus, are toll-like Receptors (TLRs). A group of them recognize virus associated patterns. TLRs are transmembrane proteins, that when bound to its ligand (PAMs: pamps pathogen-associated molecular patterns), can cause inflammatory responses and cytokine production.
In this work, cDNA from head kidney of healthy fish, asymptomatic and symptomatic (infected with IPNv) was used to quantify TLRs expression by RTqPCR.
Expressions of TLRs that recognize as ligand double-stranded RNA or unknown ligand (TLR3, 7, 8, 9, 13, 19, 21 and 22) were analyzed. In parallel we study the protein expression level of TLR22 and Myd88 by immunofluorescence head kidney and spleen.
Preliminary results indicate that exist variation in expression between healthy and infected individuals. With respect to the results obtained with TLR22, this receptor shows increased expression in symptomatic fish compared to the healthy and asymptomatic salmons.

FONDECYT-3130348, FONDAP (INCAR)-15110027.

THE *IN VITRO* EFFECT OF *TRANS*-RESVERATROL ON THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) LYMPHOCYTES PROLIFERATION AND MACROPHAGES METABOLIC AND PHAGOCYTIC ACTIVITY

S. HERMAŃSKA[1], A.K. SIWICKI[2]*, E. TERECH-MAJEWSKA[1], E. KACZOREK[1], E. SZCZUCIŃSKA[1], M. ZEMBRZUSKA[1]

[1]*University of Warmia and Mazury, Olsztyn, Poland*
[2]*Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland*

The objective of this study was to investigate the effect of *trans*-resveratrol, a potent antioxidant with anti-inflammatory and chemopreventive properties, naturally occurring in many fruits and plants on lymphocytes proliferation and also on macrophages metabolic and phagocytic activity. The aim of this study was to demonstrate the immunomodulatory effects of the compound on fish immunocompetent cells and determine the type of this interaction (immunosuppression or immunostimulation). Proliferative activity of lymphocytes was studied by MTT assay, and the respiratory burst was evaluated using the RBA test. Phagocytic killing was tested using the PKA test. The experiment have shown that *trans*-resveratrol suppressed blood B cells, while there was no significant influence on blood T lymphocytes. However, insignificant stimulatory effect occurred at the lowest concentration. In addition, the compound inhibited proliferation of T and B lymphocytes isolated from the organs. Importantly, *trans*-resveratrol caused stimulation of blood and organs macrophages phagocytic killing, and also increased the respiratory burst of macrophages isolated from organ. This substance did not significantly impact (on) cell viability, although in the case of cells isolated from the organs some augmentation was observed. These results suggest a potential use of *trans*-resveratrol as an immunomodulator of innate immunity in fish. This is particularly important, as this kind of resistance plays leading role in protecting the body against infection. In comparison, adaptive immunity is slower and also much less precise.

EXPRESSION ANALYSIS AND BIOLOGICAL ACTIVITYS OF AN ANTIMICROBIAL PEPTIDE, PISCIDIN, FROM ROCK BREAM (*OPLEGNATHUS FASCIATUS*)

J.S. BAE[1], M.A. PARK[2], S.D. HWANG[2]*, J.W. KIM[1], J.M. JEONG[1], D.H. CHO[1], C.I. PARK[1]

[1]*Department of Marine Biology and Aquaculture, Gyeongsang National University, Tongyeong, South Korea*
[2]*Aquatic Life Disease Control Division, National Fisheries Research and Development Institute, Busan, South Korea*

Many antibiotics have been used to prevent infectious diseases in the fish farms. However, bacterial resistance to conventional antibiotics is becoming more widespread. Consequently, there have been many recent attempts to find effective replacements for antibiotic use. Antimicrobial peptides (AMPs) are crucial effectors of innate immune response and are present in virtually all life forms. The AMPs typically have broad-spectrum antimicrobial activity and modulate the immune response. The piscidins, a family of AMPs in fish, share the properties of an amphipathic alpha-helical structure, low molecular weight and cationic charge at physiological pH. In this study, we identified rock bream piscidin (Rbpis) and investigated its gene expression in rock bream. The antimicrobial and cytotoxic activity was determined by the synthetic peptides based on their amino acid sequences. The ORF of Rbpis (213 bp) encoded 70 aa. Tertiary structure prediction of Rbpis showed amphipathic α-helical structure. When compared with other known AMPs amino acid sequences, the signal peptide and the antimicrobial peptide 12 domain region were conserved. Quantitative real-time PCR analysis revealed that the gene expression of Rbpis were especially high in the gill of healthy fish. After pathogens infection, Rbpis gene was down-regulated or observed no significant difference with control in the kidney and spleen, only up-regulated in the gill. Synthetic peptide of Rbpis appeared strong and broad spectrum antimicrobial activity to various bacteria in spite of weak hemolytic activity against fish erythrocytes. Rbpis is expected as very valuable potential therapeutic tools against pathogens causing lethal diseases in rock bream as *S. iniae*, other *Vibrio* sp. and RSIV.

DOSE DETERMINATION OF CHITOSAN FOR ORAL ADJUVANT IN OLIVE FLOUNDER (*PARALICHTHYS OLIVACEUS*)

M.H. JUNG*, S.M. NAM, S.J. JUNG

Department of Aqualife Medicine, Chonnam National University, Chonnam 550-749, Republic of Korea

Adjuvants are substances added to vaccines to enhance the immune responses for a given antigen. Most of the adjuvants are toxic at certain doses, and toxicity varies in different species. In this study, dose ranging studies of chitosan was carried out to determine the acceptable dosage for oral vaccination in olive flounder (*Paralichthys olivaceus*). Total amount of chitosan (3.5, 17.5 and 35mg per fish) were mixed with commercial pellet (1% body weight) and fed into flounder fingerings (10.47 ± 1.52cm, 12.66 ± 1.41g) for 10 days. AST/ALT levels in the serum were at normal range until 3 days, but high at 7 and 14 days with highest value at 35mg/fish^{-1} group. Chitosan was not lethal, however increased mucus cell numbers in the epithelium and dilated submucosal area with few inflammatory cell infiltrations at dose above 17.5mg/fish^{-1} was observed. However, there was less changes at dose of 3.5mg/fish^{-1}. Hence, chitosan found to be safe for feeding at dose of 3.5mg/fish^{-1} to olive flounder fingerlings and could be accept for oral vaccination studies. These results provide an insight for the selection of safe dose of chitosan as an adjuvant for oral vaccination.

CYTOKINE GENE EXPRESSION IN OLIVE FLOUNDER (*PARALICHTHYS OLIVACEUS*) AFTER ORAL ADMINISTRATION OF CHITOSAN

M.H. JUNG*, S.M. NAM, S.J. JUNG

Department of Aqualife Medicine, Chonnam National University, Chonnam 550-749, Republic of Korea

Viral hemorrhagic septicemia virus (VHSV) in olive flounder (*Paralichthys olivaceus*) remains an unsolved health problem in Korean aquaculture. Therefore, development of control and preventive measures against VHSV especially in small sized fish are required because of high mortality. Mucosal immune responses were assesed by analysing cytokine gene expression levels in the intestine of olive flounder to develop effective and safe oral vaccine using chitosan as an adjuvant. Total amount of chitosan (1.6mg per fish) were mixed with commercial pellet (1% body weight) and fed into flounder fingerings (6.47 ± 1.12cm, 6.00 ± 1.23g) for 7 days. In the intestine, high levels of IL 8 and CD4 expression (4.79- and 2.73-fold, respectively) were observed at 4 days post administration (dpa) and then reduced (1,92- and 1.11-fold, respectively) at 7 dpa. IL-1β expression was increased with the time showing 0.35-, 1.25- and 3.59-fold at 1 d, 4 d and 7 dpa, respectively. Basal or down regulated expression of IL 6, TNFα and IFNγ were observed at 1 d (0.66-, 1.21- and 1.25-fold), 4 d (0.58-, 1.57- and 1.30-fold) and 7 dpa (0.62-, 1.30- and 0.38-fold). These data shows that chitosan has pro-inflammatory as well as anti-inflammatory responses, indicating that it has immune modulatory effect, which will be enhanced immune responses when virus get infected. Additionally, higher expression of CD4 mRNA indicates the activation of $CD4^+$ immune cells in the early phase of immune responses.

EFFECTS OF REARING DENSITY ON TIGER PUFFER (*TAKIFUGU RUBRIPES*) AND JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS*) IMMUNE SYSTEM

K. KOBAYASHI[1]*, H. USHIO[2], I. HIRONO[1], H. KONDO[1]

[1]*Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Tokyo, Japan*
[2]*Laboratory of Marine Biochemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*

Fish in farms are generally kept under conditions of higher rearing density than those in natural condition. Chronic high rearing density causes stress in fish. In mammals, chronic stress induces the suppression of immune system. In fish, however, there are only a few reports about the relationship between chronic stress derived from high rearing density and immune system. In this study, to understand the effects of rearing density on tiger puffer (*Takifugu rubripes*) and Japanese flounder (*Paralichthys olivaceus*) immune system, we conducted gene expression profiling for stress- and immune-related genes using quantitative PCR (qPCR).
Tiger puffer and Japanese flounder were kept in high and low rearing density condition for 15 and 30 days. Brain, liver and spleen were collected from fish of each group. Total RNA was extracted and cDNA was synthesized. The mRNA levels of stress-related and immune-related genes were analyzed using qPCR.
In tiger puffer, the mRNA levels of heat-shock protein (HSP) 70 and 90 gene in high density group were significantly higher than low density group. On the other hand, the mRNA levels of corticotropin-releasing hormone receptor gene were significantly lower in the high density group. In Japanese flounder, the mRNA levels of HSP70 in high rearing density group also higher than low density group. In tiger puffer, the mRNA levels of immunoglobulin M, CD4, CD8 and transforming growth factor β, genes were significantly lower than the low density group. In Japanese flounder, however the mRNA levels of all immune-related genes were not significantly changed. These results suggest that although rearing density affects stress responses and immune system of fish, these effects might vary in a species dependent manner.

IDENTIFICATION OF THE GENES INVOLVED IN ANTIGEN PRESENTATION AND T-CELL DEVELOPMENT IN JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS*) TO EVALUATE THE VACCINE EFFICACY

H. KONDO*, S. AOKA, N. KANESHIGE, I. HIRONO

Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Japan

Vaccination is a promising strategy to protect fish from infectious diseases in aquaculture. The effects of vaccines rely on the host's adaptive immunity, which is classified into cellular and humoral immunity. The components of vaccines are to be antigens, which are taken up by antigen presenting cells. The antigens are then processed and represented on the cell surface. This process involves two pathways for presentation of the exogenous (e.g. bacteria) and endogenous (e.g. virus) antigens to major histocompatibility complex (MHC) class I and II, respectively. The antigens are recognized by T-cells, which are differentiated to certain types of cell population. The balance between the cellular and humoral adaptive immunity are dependent on the pathway of antigen presentation and the types of T-cells differentiated.
Transporter associated with antigen processing (TAP) and TAP-associated glycoprotein (tapasin) interact with MHC class I to transport the antigenic peptides across the endoplasmic reticulum membrane. In mammals, the genes encoding TAP and tapasin are up-regulated by interferon γ, which activates MHC class I antigen processing and presentation. On the other hand, two transcription factors, T-bet and GATA-3 are the master regulator of T-helper 1 and 2, which are involved in the development of cellular and humoral adaptive immunity, respectively.
In this study, we identified homologues encoding TAP, tapasin, T-bet and GATA-3 in Japanese flounder, and investigated their expression profiles after the intra-peritoneal injection of formalin-killed cells (FKC) of bacteria. The homologous genes encoding TAP1, TAP2, 2-types of tapasin like molecules, T-bet and GATA-3 were identified in EST datasets obtained by next-generation sequencing. They showed high amino acid identities to those in the other vertebrates. The genes were detected in the all organs, especially in the spleen and kidney. Their expression profiles after FKC treatments are now being investigated.

IDENTIFICATION OF THE GENES INVOLVED IN ANTIGEN PRESENTATION AND T-CELL DEVELOPMENT IN JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS*) TO EVALUATE THE VACCINE EFFICACY

H. KONDO*, S. AOKA, N. KANESHIGE, I. HIRONO

Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Japan

Vaccination is a promising strategy to protect fish from infectious diseases in aquaculture. The effects of vaccines rely on the host's adaptive immunity, which is classified into cellular and humoral immunity. The components of vaccines are to be antigens, which are taken up by antigen presenting cells. The antigens are then processed and represented on the cell surface. This process involves two pathways for presentation of the exogenous (e.g. bacteria) and endogenous (e.g. virus) antigens to major histocompatibility complex (MHC) class I and II, respectively. The antigens are recognized by T-cells, which are differentiated to certain types of cell population. The balance between the cellular and humoral adaptive immunity are dependent on the pathway of antigen presentation and the types of T-cells differentiated.
Transporter associated with antigen processing (TAP) and TAP-associated glycoprotein (tapasin) interact with MHC class I to transport the antigenic peptides across the endoplasmic reticulum membrane. In mammals, the genes encoding TAP and tapasin are up-regulated by interferon γ, which activates MHC class I antigen processing and presentation. On the other hand, two transcription factors, T-bet and GATA-3 are the master regulator of T-helper 1 and 2, which are involved in the development of cellular and humoral adaptive immunity, respectively.
In this study, we identified homologues encoding TAP, tapasin, T-bet and GATA-3 in Japanese flounder, and investigated their expression profiles after the intra-peritoneal injection of formalin-killed cells (FKC) of bacteria. The homologous genes encoding TAP1, TAP2, 2-types of tapasin like molecules, T-bet and GATA-3 were identified in EST datasets obtained by next-generation sequencing. They showed high amino acid identities to those in the other vertebrates. The genes were detected in the all organs, especially in the spleen and kidney. Their expression profiles after FKC treatments are now being investigated.

DISTRIBUTION OF T CELLS THROUGHOUT RAINBOW TROUT SKIN AND RESPONSIVENESS TO VIRAL INFECTION

E. LEAL, A.G. GRANJA, C. TAFALLA*

Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos 28130, Madrid, Spain

In fish, the skin constitutes the first line of defense against water-borne pathogens. Because teleost skin is not keratinized, skin cells are in close contact with the water medium and consequently can immediately react to pathogen exposure. Despite this, there is a great lack of information regarding a phenotypic and functional characterization of the skin associated lymphoid tissue (SALT). Furthermore, whether immune components of the skin are homogeneously distributed through the surface of the fish is still unknown. In the current work, we have analyzed the transcription of several immune genes throughout different rainbow trout (*Oncorhynchus mykiss*) skin areas. We found that while immunoglobulin and chemokine gene transcription levels were evenly distributed through the cell surface, the expression of genes related to T cell function such as T cell receptor a (TCRa), TCRg, CD3, CD4, CD8 and Eomes was significantly higher in anterior areas closer to the gills. In agreement with these results, immunohistochemical analysis revealed a higher concentration of $CD3^+$ cells in anterior skin areas in comparison to posterior skin sections. Finally, when fish were exposed to a viral pathogen in the water, immune genes related to T helper lymphocyte activity (CD3, CD4 and TCRa) were significantly up-regulated in the skin in response to the virus; however the differences between anterior and posterior sections were still maintained. These results highlight the importance of skin T cell responses in antiviral defense in fish and demonstrate for the first time that T cells are not homogeneously distributed throughout the teleost skin.

CHARACTERIZATION OF IMMUNE MOLECULAR MARKERS TO IDENTIFY TRAITS OF IMMUNE COMPETENCE IN THE NORTHERN SCALLOP *ARGOPECTEN PURPURATUS*

D. OYANEDEL[1], P. SCHMITT[1], R. GONZÁLEZ[2], K. BROKORDT[2], L. MERCADO[1]*

[1]*Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología. Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[2]*Centro de Estudios Avanzados en Zonas Aridas (CEAZA), Universidad Católica del Norte, Coquimbo, Chile*

The Northern scallop aquaculture in Chile has faced recurrent episodes of larval mortalities that has been attributed to bacterial infections in the last decades. This has motivated the increase of the knowledge on the immune response and the development of genetic improvement programs on this mollusk. These programs aim to identify relevant and heritable immune traits for their use on selective breeding. In this context, the characterization of immune-related genes in *Argopecten purpuratus* becomes essential to identify potential immunological markers. Like all invertebrates, the immune response of scallops depends exclusively on innate immune mechanisms. This response is mediated by cellular and humoral components, such as phagocytosis and production of antimicrobial peptides, and oxygen and nitrogen radicals. A crosstalk between oxygen radicals (ROS) and the transcription factor NF-kB has been reported besides the ROS classical microbe-destroying activity. This suggests a modulatory role of this metabolite on enhancing the production of antimicrobial molecules as result of a complementary activation of the NF-kB pathway. Therefore, in this work we aimed to characterize immune-related genes from the Northern scallop and to elucidate the potential interaction between ROS and antimicrobial peptide production. For this, a set of genes with a putative role on the ROS-antimicrobial peptide crosstalk were characterized. The encoding genes for catalase, peroxiredoxin and the intracellular Cu/Zn SOD as components of the antioxidant response, the antimicrobial peptide Big-defensin, and the regulators of NF-kB pathway calcineurin A and inhibitor kappa beta IκB were identified by RT-PCR using degenerate primers designed from available sequences from other scallop species. The relative expression of these genes and the production of oxygen radicals in response to different microbe associated molecular patterns (MAMPs) will be evaluated on primary cultures of hemocytes by qPCR and dichlorofluorescin diacetate, respectively. Next, ROS production will be inhibited by specific scavengers and the effect on the expression of antimicrobial effectors and regulators of NF-kB pathway will be evaluated. The results will allow to propose molecular immune markers to establish the specific redox state that enhance the expression of antimicrobial effectors, resulting in an improvement of the scallop immune response.

FONDECYT 1140849.

SEASONAL CHANGES OF IMMUNE PARAMETERS IN RAINBOW TROUT (*ONCORRHYNCHUS MYKISS*), BROOK TROUT (*SALVELINUS FONTINALIS*) AND HYBRID OF BROOK TROUT AND ARCTIC CHARR (*S. FONTINALIS* X *S. ALPINUS*)

I. PAPEŽÍKOVÁ[1]*, J. MAREŠ[2], L. VOJTEK[3], P. HYRŠL[3], Z. SOUKUPOVÁ[1], M. PALÍKOVÁ[1]

[1]*University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic*
[2]*Mendel university Brno, Brno, Czech Republic*
[3]*Masaryk Uuniversity Brno, Brno, Czech Republic*

Immune system of fish includes both non specific (innate) and specific (adaptive) immune mechanisms which afford protection against invading pathogens. Fish immune system function is dependent on many factors, mainly on water temperature. It is widely accepted that higher temperature enhances immune response while low temperature leads to suppression of immune system. The aim of this work was to follow up seasonal changes of immune parameters in three species of salmonid fish.
Samples of fish (eight individuals of each species) were taken at five time intervals – at the end of April, at the beginning of July, at the end of August, at the beginning of November and at the beginning of February. Blood samples were taken and used for assessment of leukocyte counts, complement activity, oxidative burst of phagocytes and total plasma immunoglobuline concentration. Sample of skin mucus was used for measurement of lysozyme levels. Water temperature was recorded in every sampling.
Leukocyte counts (both phagocytes and lymphocytes) peaked in summer months and dropped during winter in all species. Lysozyme in skin mucus reached highest level at the end of April, then the level continuously declined up to zero value measured out in February. Oxidative burst and complement activity did not exert distinct seasonal dynamics. Immunoglobulin levels markedly increased in July in brook trout and hybrid, but not in rainbow trout where the levels remained low and significant increase was found as late as at the end of August. On the other side, high immunoglobuline levels still persisted at the beginning of November in rainbow trout, while in hybrids and brook trout the levels already declined at this time point. From the three examined fish species, the most pronounced seasonal changes of immune parameters were found in rainbow trout and lowest in hybrids. Although it could be expected that immune system of fish will work most intensively in highest summer temperatures, our results show that the dynamics of most of the measured parameters did not exactly correspond to temperature changes.

This work was supported by project MZe NAZV (QJ1510077).

EVIDENCE OF KINOCIDIN ACTIVITY IN CHEMOKINE IL-8 FROM RAINBOW TROUT

P.A. SANTANA[1, 2]*, F. GUZMÁN[2], L.A. MERCADO[1, 2]

[1]*Grupo de Marcadores Inmunológicos. Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[2]*Núcleo Biotecnológico de Curauma (NBC), Valparaíso, Chile*

Antimicrobial peptides (AMPs), are one of the major components of the innate defenses in protecting from such infections. They usually have the common characteristics: small peptide (12–60 aa), strong cationic, heat-stable, no drug fastness and no effect on eukaryotic cell. In higher vertebrates, bioinformatic analyses have led to the identification of structural themes that unify many classes of antimicrobial peptides that previously thought unrelated. The AMPs stabilized by disulfide bridges, are characterized by having in some cases a C-terminal α-helical domain with periodic charged and hydrophobic amino acid composition conferring its microbicidal activity and the γ -core motif with its discrete and complementary functions, interposing the N- and C-terminal domains. Chemokines (CXC) exhibit those domains and have been reported to have direct microbicidal activity. Hence, the term kinocidin has been applied to encompass chemokines with direct microbicidal activity. In lower vertebrates, the CXC interleukin-8 (IL-8), has a predominantly chemotactic function; however, it is not known if it possesses antimicrobial activity. In this work it was found that C-terminal α-helix domain of trout IL-8 possess antimicrobial activity. Through an *in silico* analysis of the primary structure of IL-8 from *Oncorhynchus mykiss*, was evidenced that γ motif was present, as in the vast majority of kinocidins. We synthetized the α-helix domain of IL-8 (αIL-8) by solid phase chemistry synthesis and showed a tendency to form α-helix conformation as revealed by circular dichroism. Moreover, the recombinant IL-8 was obtained and partial acid hydrolysis was made. This assay showed that fragment corresponding to C-terminal α-helix of IL-8 was released. Finally the peptide αIL-8 showed antimicrobial activity against *Pseudomonas aeruginosa.* These results suggest that given the structural and functional characteristics of IL-8, it is possible that alpha helix domain of this chemokine possess antimicrobial activity. A molecule type kinocidin like IL-8, which possess both antimicrobial and chemotactic characteristics, makes it more attractive for application in the productive sector of salmon.

THE FIRST CHARACTERIZATION OF AN IFN-GAMMA GENE IN TURBOT (*SCOPHTHALMUS MAXIMUS*) REVEALS INTERESTING QUESTIONS ABOUT ITS IMMUNOMODULATORY PROPERTIES

P. PEREIRO*, G. FORN-CUNI, A. FIGUERAS, B. NOVOA

Instituto de Investigaciones Marinas (IIM), CSIC, Vigo, Spain

Interferons (IFNs) are a family of multifunctional cytokines representing the first defensive line against viral infections among other immune relevant functions. These proteins are produced in response to different pathogen or pathogen-associated molecular patterns (PAMPs) via the activation of different signaling pathways. In fish, two subfamilies of IFNs were established in basis to different structural and functional properties (type I and type II IFNs). Type I IFN subfamily comprises a group of typically antiviral proteins, and a variable number of type I IFNs was described in different fish species. Indeed, it has been shown that type I IFNs from the same teleost species can possess different properties and capabilities, suggesting in some cases complementary or specialised roles, as was previously observed in turbot. IFNγ (type II IFN) is a markedly different cytokine than type I IFNs, possessing some ability to interfere with viral infections but being mainly an immunomodulator. Unlike mammals, it has been shown that some bony fish have two type II interferons, IFN-gamma (IFNγ) and IFN-gamma related (IFNγrel), whose pro-inflammatory functions have not been fully characterized. While exploring the turbot genome we found only one type II IFN, and this corresponded to IFNγ.

In the current work, we have characterised for the first time a type II IFN gene in turbot. To get insights into its functions, we analysed its constitutive expression and gene modulation after viral (VHSV) and bacterial (*Aeromonas salmonicida*) challenge. Moreover, we tested the induction of IFNγ by type I IFNs as well as the bioactivity of IFNγ measuring the induction of specific immune genes and the protection capabilities against infection. Interestingly, no protection was observed against both bacterial and viral infections. Nevertheless, some interesting questions were observed, especially concerning the regulation of the macrophages during VHSV challenge.

STUDY OF THE IMMUNE RESPONSE AFTER *AEROMONAS* SPP. CHALLENGE IN TURBOT (*SCOPHTHALMUS MAXIMUS*) AND ZEBRAFISH (*DANIO RERIO*)

P.R. SARACENI, P. PEREIRO*, A. ROMERO, A. FIGUERAS, B. NOVOA

Instituto de Investigaciones Marinas (IIM), CSIC, Vigo, Spain

Aeromonas salmonicida subsp. *salmonicida* is a Gram-negative bacterium that is the causative agent of furunculosis, a systemic disease in the salmonid farming industry. Moreover, many other species have shown to be susceptible to this pathogen, including turbot. In this work, the immune response to this pathogen, mainly the inflammation process, has been analyzed in turbot. A fast but non-maintained induction of TNF-alpha and IL-1 beta was observed. However, other important molecules involved in the defense against the bacteria showed a slower response, but it was more sustained over time. This kind of analysis is useful in order to know the genes implicated in the defense mechanism, but it is difficult to obtain more information using turbot as model for studying the infections caused by *Aeromonas* spp. The advantages of the zebrafish as infection model could contribute to a better understanding of the pathogen-host interaction during the infection process. For that reason, the establishment of models of infection in zebrafish larvae to study the pathogenesis of *Aeromonas hydrophila* AH-1 septicaemic strain was conducted. In this work we established different models of infection of *A. hydrophila* in zebrafish larvae: microinjection (using different routes) and bath infection (in intact and injured larvae). In addition to this, we used two transgenic lines TG(mpx:gfp) and TG(IL-1b:gfp) expressing fluorescent neutrophils and IL-1b respectively, to characterize *in vivo* the neutrophil-mediated inflammatory response and the expression pattern of IL-1b after *A. hydrophila* challenge. The expression of several immune genes was quite similar to that observed in turbot after *A. salmonicida* challenge.

TRANSCRIPTOME ANALYSIS OF THE MAIN IMMUNE PATHWAYS AFTER VHSV INFECTION IN TURBOT (*SCOPHTHALMUS MAXIMUS*)

P. PEREIRO*, B. NOVOA, A. FIGUERAS

Instituto de Investigaciones Marinas (IIM), CSIC, Vigo, Spain

Viral haemorrhagic septicaemia virus (VHSV) is a fish pathogen belonging to the genus Novirhabdovirus, within the family Rhabdoviridae. This etiological agent causes an important viral disease affecting rainbow trout *Oncorhyncus mykiss* and other salmonids, but VHSV outbreaks have been detected in other marine farmed fish species such as turbot. Nowadays, the culture of this flatfish is well-established being a very important commercial species for the aquaculture industry in Europe and Asia. However, infectious diseases are one of the most relevant limiting factors, causing severe economic losses in many cases. Neither vaccines nor therapeutic treatments are commercially available for this disease. Therefore, the knowledge about the main molecules implicated in the immune response against this pathogen is a very important question in order to find selective markers for breeding programs as well as antiviral treatments and/or vaccine adjuvants.

We have used a specific microarray highly enriched in antiviral sequences to carry out the transcriptomic study associated to VHSV infection. The differential gene expression pattern in response to the virus with regard to non-stimulated turbot was analysed in head kidney at 8, 24 and 72 hours post-challenge. In order to assess the viral replication success of VHSV strain UK-860/94 in head kidney the expression of the five viral genes was also tested. A Gene Ontology (GO) classification of biological processes at the 2nd level of the modulated genes after VHSV infection revealed a time-increasing representation of genes with a direct implication in immunity. Moreover, the enrichment analyses led us to identify those overrepresented biological processes at each sampling point. Genes implicated in the Toll-like receptor signalling pathway, IFN inducible/regulatory proteins, numerous sequences implicated in apoptosis and cytotoxic pathways, MHC class I antigens, as well as complement and coagulation cascades among others were analysed using hierarchical clustering.

EFFICACY OF ICTHIOVAC® VR AGAINST *LISTONELLA ANGUILLARUM* SEROVAR O2 IN EUROPEAN SEA BASS FRY AND JUVENILES

M.C. RUIZ, A. CALLOL, S. COLOMER, D, VENDRELL, M. PERELLÓ*, R. ROBLES

HIPRA, Amer (Girona), Spain

European sea bass (*Dicentrarchus labrax*) is a marine fish species with a high commercial value, especially in the Mediterranean countries and Europe. Since 1992, aquaculture production of this species has widely overcome wild stock captures [1]. Several pathologies act as a bottleneck during aquaculture production process, including bacteria, viruses, parasites and fungi. Hence, to control rearing-associated problems such as pathologies have become indispensable to ensure a good production and avoid economic loses. Vibriosis provoked by *Listonella* (*Vibrio*) *anguillarum* is responsible of important outbreaks affecting sea bass production farms. It causes a hemorrhagic septicaemia leading on high morbidity and mortality rates [2]. Therefore, development and use of preventive strategies can evade these associated mortalities.
ICTHIOVAC® VR is an inactivated vaccine against Vibriosis initially developed for turbot. This study is intended to demonstrate the efficacy of ICTHIOVAC® VR, in preventing mortality caused by *Vibrio (Listonella) anguillarum*, serotype O2, in Sea bass. Fish were vaccinated by two recommended size-dependent routes: dip immersion for fry sized 0.5 g to 2 g, and intracelomic injection for juveniles from 15 g onwards, and challenged with one *L. anguillarum* O2 virulent strain.
The RPS values obtained were of 64 % in 0.5-2 g fry vaccinated by dip immersion and of 77 % in 15-g juveniles vaccinated by intracelomic injection. Deaths occurred between 48 and 96 hours after challenge, reaching mortality rates ranging between 60% and 98% in control groups
The results obtained demonstrate that ICTHIOVAC® VR is effective in conferring immunity to sea bass from 0.5 g to 15 g against virulent *Vibrio (Listonella) anguillarum* O2 strain by two different size-dependent routes, by dip immersion and by intracelomic injection.

[1] FAO Fisheries and Aquaculture. Fishery Statistical Collections. On line resource: http://www.fao.org/fishery/statistics.
[2] Frans I., Michiels C. W., Bossier P., Willems K.A., Lievens B. and Rediers H. (2011) Vibrio anguillarum as a Fish pathogen: virulence factors, diagnosis and prevention. Journal of Fish Diseases 34, 643-661.

EFFICACY OF ICTHIOVAC® VR AGAINST *LISTONELLA ANGUILLARUM* SEROVAR O1 IN EUROPEAN SEA BASS FRY AND JUVENILES

M.C. RUIZ, A. CALLOL, S. COLOMER, D, VENDRELL, M. PERELLÓ*, R. ROBLES

HIPRA, Amer (Girona), Spain

European sea bass (*Dicentrarchus labrax*) is a marine fish species with a high commercial value, especially in the Mediterranean countries and Europe. Since 1992, aquaculture production of this species has widely overcome wild stock captures [1]. Several pathologies act as a bottleneck during aquaculture production process, including bacteria, viruses, parasites and fungi. Hence, to control rearing-associated problems such as pathologies have become indispensable to ensure a good production and avoid economic loses. Vibriosis provoked by *Listonella* (*Vibrio*) *anguillarum* is responsible of important outbreaks affecting sea bass production farms. It causes a hemorrhagic septicaemia leading on high morbidity and mortality rates [2]. Therefore, development and use of preventive strategies can evade these associated mortalities.
ICTHIOVAC® VR is an inactivated vaccine against Vibriosis initially developed for turbot. This study is intended to demonstrate the efficacy of ICTHIOVAC® VR, in preventing mortality caused by *Vibrio (Listonella) anguillarum,* serotype O1, in Sea bass. Fish were vaccinated by the two recommended size-dependent routes: dip/bath immersion for fry sized 0.5 g to 2 g, and intracelomic injection for juveniles from 15 g onwards, and challenged with one *L. anguillarum* O1 virulent strain.
The RPS values obtained were of 80.6% and 73% in 0.5-2 g fry vaccinated by dip and bath immersion respectively and of 100% was obtained in 15-g juveniles vaccinated by intracelomic injection. Deaths occurred between 48 and 96 hours after challenge, reaching mortality rates ranging between 68-97 % in control groups
The results obtained demonstrate that ICTHIOVAC® VR is effective in conferring immunity to Sea bass from 0.5 g to 15 g against virulent *Vibrio (Listonella) anguillarum* O1 strain by two different size-dependent routes, by dip/bath immersion and by intracelomic injection.

[1] FAO Fisheries and Aquaculture. Fishery Statistical Collections. On line resource: http://www.fao.org/fishery/statistics.
[2] Frans I., Michiels C. W., Bossier P., Willems K.A., Lievens B. and Rediers H. (2011) Vibrio anguillarum as a Fish pathogen: virulence factors, diagnosis and prevention. Journal of Fish Diseases 34, 643-661.

ASSESSMENT OF IMMUNE RESPONSE CAPABILITY IN RAINBOW TROUT LARVAE VIA IL-8 /IL-8R

P.A. SANTANA[1, 2]*, L.J. CORTÉS[1], S.R. SARIEGO[1], F. GUZMÁN[2], L. MERCADO[1, 2]

[1]*Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[2]*Núcleo Biotecnológico de Curauma (NBC), Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*

The salmonids are affected by diverse pathogens. At date immunoprophylactic methods are used in larval and adult stages in order to strengthen the immune system and prevent infections caused by pathogens. To understand the effect of modulators on fish in early stages is necessary to study effectors and regulatory molecules of the immune system. The chemokines and their receptors play remarkably varied and potent roles in immunobiology and the knowledge of these molecules is still limited in fish. Interleukin 8 (IL-8) is a chemokine produced by several types of cells and plays an important role during the inflammatory process. In mammals IL-8 is known act through of two different receptors named CXCR1 and CXCR2, but in Rainbow trout only one IL-8 receptor (IL-8R) has been identified. In trout larvae IL-8 and IL-8R were evaluated at the transcriptional level; however the protein levels have not been evidenced. The aim of this work was characterize the ability of immune response in early stages of trout challenged with LPS through detection of IL-8 and IL-8R. We demonstrate that LPS-challenged for 8 hours induces the expression of IL-8 and IL-8R at transcriptional level. Immunological methods were used for analyses of protein expression in larvae of 19 days post hatch. Polyclonal antibodies using synthetic epítope-peptides were generated against IL-8 and IL-8R. The presence of IL8 and IL-8R was detected by ELISA, demonstrated an increase in the expression of these molecules. This result suggests the activation of the immune response. Finally, IL-8R was colocalizated with its ligand by immunofluorescence in skin and gills. Our results suggest that cells of the innate immune system of larvae challenged with LPS express on their surface receptors such as IL-8R and chemokines such as IL-8 to perform a protective immune response. The results obtained in this work suggest that IL8 and their receptor can be used as potential indicators of disease susceptibility.

MOLECULAR CLONING AND FUNCATIONAL ANALYSIS OF SERUM AMYLOID P COMPONENT IN ROCK BREAM, *OPLEGNATHUS FASCIATUS*

S.D. HWANG[1]*, M.Y. CHO[1], K.I. KIM[1], C.I. PARK[2], M.A. PARK[1]

[1]*Aquatic Life Disease Control Division, National Fisheries Research and Development Institute, Busan, Korea*
[2]*Department of Marine Biology and Aquaculture, Gyeongsang National University, Tongyoung, Korea*

It has been widely known that the serum amyloid P component (SAP) serves to recognise a wide range of exogenous pathogenic substances and activate a complementary pathway to pathogen clearance in the pathogen study of mammals. On the contrary, there has been little information on the expression profiles and functions of SAP for fish. To determine potential roles of SAP in the fish immune system, we identified and investigated the molecular characterization of SAP (RbSAP) in rock bream (*Oplegnathus fasciatus*). We also determined gene expression profiles of RbSAP both in healthy fish and infected fish. The length of the coding region in RbSAP cDNA was 687 bp, which encodes 228 amino acid residues. RbSAP gene consists of a signal peptide and a pentraxin domain. According to phylogenetic analysis, RbSAP gene was classified with other known fish SAPs. RbSAP expression was high in the liver of healthy rock bream. Pathogen exposure led to differential patterns of gene expression in the tissues. RbSAP gene was highly induced in the spleen when injected with *Streptococcus iniae* and red sea bream iridovirus (RSIV), and in the liver when injected with *Edwardsiella tarda* and RSIV. To understand the functional characterization of SAP in fish, we investigated bacterial growth using a recombinant RbSAP protein. The result indicated that Ca^{2+} impacted bacterial growth by bacteria-type-specific activation: a high concentration of the recombinant RbSAP without Ca^{2+} inhibited the growth of *S. iniae* while a high concentration of the recombinant RbSAP with Ca^{2+} barely inhibited the growth of *E. tarda.* Similar findings were observed for bacterial agglutination by the recombinant RbSAP. These research results suggest that RbSAP plays an important role in the immune response against pathogens.

CHRONIC STRESS DOWN REGULATED THE PATTERN RECOGNITION RECEPTORS (PRRS) EXPRESSION IN THE SKELETAL MUSCLE OF THE FINE FLOUNDER

C. VALENZUELA[1, 2, 3]*, R. ZULOAGA[1, 2, 3], J.A. VALDES[1, 2, 3], A. MOLINA[1, 2, 3]

[1]*Laboratorio de Biotecnología Molecular, Universidad Andrés Bello, Santiago, Chile*
[2]*Interdisciplinary Center for Aquaculture Research (INCAR), Concepción, Chile*
[3]*Centro de Investigación Marina de Quintay (CIMARQ), Valparaiso, Chile*

In mammals, stress has a negative effect on the immune system generating a susceptibility to pathogens and diseases. The key sensors involved in the detection of pathogens and foreign molecules are the pattern recognition receptors (PRRs), which recognize a variety of pathogens associated molecular patterns (PAMPs) such as LPS, single-stranded RNA, double stranded RNA, peptidoglycan, viral nucleotides, among others. Between PRRs we found Toll-like receptors (TLR), NOD-like receptors (NLRs) and the retinoic acid inducible gene I (RIG-I) like receptors (RLRs). In fish, have been characterized several TLR, and a few NLR and RLR. The studies of these receptors have been addressed just in organs with potent immune response, as the head kidney, spleen and gills. However, in skeletal muscle, which represents more than 50% of the body, the ability to trigger an immune response and the effect of stress in this reaction has not been studied in detail.

In this context, the aim of this study was to evaluate the expression of PRRs under confinement as a classic stress farming condition, to make a descriptive analysis of PAMPs detection machinery in the skeletal muscle of Chilean flounder. The fine flounder (*P. adspersus*) were subjected to 4 and 7 weeks of confinement inducing a stress response, collecting skeletal muscle samples from control and stressed fish at each experimental time. To confirm the stress condition, cortisol plasma level was measured by ELISA and relative expression of glucocorticoid receptor (gr1 and 2) was evaluated through qPCR. Thus, TLRs, NLR (*nod1* and *nlrc3*) and RLR (*mda5* and *lpg2*) gene expression were analyzed, showing a clear trend to decrease of all receptors mRNA levels during the entire trial.

These preliminary results suggest that the stress response, triggered by the high population density, has a negative effect on the expression of the receptors responsible for pathogen recognition in skeletal muscle of fine flounder, which could explain the susceptibility to pathogens and disease that generates stress in fish farming.

CYTOKINES EXPRESSION IN SAF-1 CELL LINE INFECTED WITH *PHOTOBACTERIUM DAMSELAE* SUBSP. *PISCICIDA*

J. VEGA, D. PADILLA, L. ROMÁN, M.S. DÉNIZ, I. ROSARIO, B. ACOSTA*, F. EL AAMRI, F. REAL

**Instituto Universitario de Sanidad Animal (IUSA), University of Las Palmas de Gran Canaria, Canary Islands, Spain*

Photobacterium damselae subspecies *piscicida* is an important pathogen of many marine fish species cultured in the United States, Japan, Europe and the Mediterranean. Fibroblasts have shown to be an immune competent cell type in mammals. However, little is known about the immunological functions of this cell type in lower vertebrates, like fish species. Measuring cytokines expression is a tool to determinate immune response.
SAF-1 cell line, derived from *Sparus aurata* fin, was infected by *Photobacterium damselae* subsp. *piscicida* at a multiplicity of infection (MOI) of 10~1, 25~1, 50~1 and 100~1 (bacteria/fish cell ratio). In order to determinate the MOI that produces the maximum expression for further assays, Mx, IL-1β, IL-IR2, TNF-α and COX-2 expression in SAF-1 cells infected was determined by real time PCR. After 4 hours of infection, the expression of IL-1β, COX-2, IL-IR2 and TNF-α was the highest level at a MOI of 10~1.The expression of Mx reached the top at a MOI of 100~1. These results showed the MOI used affects immune response on SAF-1 fibroblastic cell line and we decided to use the lowest MOI (10~1), which showed the better preliminary result, in a more complex assay. In this study, SAF-1 cell line was infected by five different strains of *Photobacterium damselae* subsp. *piscicida* (C2, 94/99, ATCC17911, DI-21 and PP3). Cytokines expression was determined by real time PCR after 90 minutes, 4, 12, 24 and 48 hours post-infection. Results showed different expression levels depending on the strain, time and cytokine studied. Proinflammatory cytokines TNF-α and COX-2 expression were especially up-regulating. These results suggest that *Sparus aurata* fibroblasts are able to contribute significantly to immune reactions in concert with the traditional immune cells.

MOLECULE CHARACTERIZATION OF CXC CHEMOKINE LIGAND 12 FROM ORANGE-SPOTTED GROUPER (*EPINEPHELUS COIOIDES*) IN RESPONSE TO NODAVIRUS INFECTION

W. CHENG-HSIU[1, 2], L. CHIN-FENG[1, 2], L. HAO-PING[1], C. YOUNG-MAO[1, 2, 3], C. TZONG-YUEH[1, 2, 3, 4, 5]*

[1]*Institute of Biotechnology, National Cheng Kung University, Tainan, Taiwan*
[2]*Translational Center for Marine Biotechnology, National Cheng Kung University, Tainan, Taiwan*
[3]*Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan, Taiwan*
[4]*University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan*
[5]*Research Center of Ocean Environment and Technology, National Cheng Kung University, Tainan, Taiwan*

CXC chemokine ligand 12 (CXCL12) is an inflammatory chemokine of the immune system. The chemokine CXCL12 and its receptor, CXCR4, have believed to be a specific ligand-receptor pair which is involved in several physiological and pathophysiological processes. In this study, we attempted to clone and characterize the CXCL12 gene from *Epinephelus coioides* (*osgCXCL12*). The open reading frame of *gCXCL12* consisted of 98 amino acid residues with an estimated molecular mass of 11.27 kDa. Reverse transcription polymerase chain reaction (RT-PCR) and real-time PCR were utilized to examine *osgCXCL12* expression levels in different tissues. Our results suggest that *osg*CXCL12 was constitutively expressed in several tissues (the fin, gill, liver, and spleen; $p < 0.01$) with especially high expression in the head kidney ($p < 0.01$). Furthermore, time-course analysis of *osgCXCL12* and *osgCXCR4* (CXCR4 from *E. coioides*) expression levels in nervous necrosis virus (NNV) infected groupers revealed a significant increase after 12 and 24 h of challenge with NNV ($p < 0.05$), respectively. The *osgCXCR4* gene expression level also increased with NNV in a time-dependent manner until post-infection ($p < 0.05$). Besides, the immune related gene expression level of different development stages of *E. coioides* (0-28 day) was also evaluated by real time PCR. These data provide valuable information for further exploration of groupers chemokine signaling pathways and their roles in immune responses to virus infection.

IMMUNE CHARACTERIZATION OF NOVEL PARASITE ANTIGEN BIOMARKERS FOR PROLIFERATIVE KIDNEY DISEASE IN SALMONIDS

S. YOON[1]*, Z. QI[2], A. ALNABULSI[3], C. SECOMBES[1], J. HOLLAND[1]

[1]*Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, UK*
[2]*Yancheng Institute of Technology, Yancheng, China*
[3]*Vertebrate Antibodies Ltd, University of Aberdeen, Aberdeen, UK*

Proliferative kidney disease (PKD), caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, is one of the most economically damaging diseases to the UK trout aquaculture industry. Fish recovering from PKD develop protective immunity to subsequent parasite challenge providing the impetus for vaccine development. Our overall aim is to identify and test selected parasite antigens as vaccine targets and to develop novel anti *T. bryosalmonae* monoclonal antibodies (moAb) to use as biomarkers of infection. From our expression library immunization (ELI) studies, we selected individual parasite molecules to be tested as DNA vaccines, including immune related molecules (eg. tetraspanin and lectin-like molecules), antigens known to elicit protection in other host-parasite interactions, and unknown putative surface-expressed molecules. Preliminary studies have been undertaken to investigate *in vitro* and *in vivo* expression of candidate parasite genes cloned into a modified version of the pcDNA6 vector. So far, DNA vaccines encoding *T. bryosalmonae* peroxiredoxin and an unknown putative surface-expressed antigen (P14G8) have been examined utilizing codon optimized recombinant genes to maximize translational efficiency. Fish were injected intramuscularly and sampled 3 and 7 days post injection. Transgene expression was detected in skin/muscle tissue by Western blotting using anti-V5 and gene specific moAb. These moAb have been shown to have high affinity to target proteins derived from; transfected cells, recombinant protein, and *T. bryosalmonae* infected trout kidney tissues. *In vivo* detection of selected parasite genes following intramuscular immunization is an important prerequisite in testing DNA vaccine candidates that will help towards the future development of a PKD vaccine.

EOSINOPHILIC GRANULOCYTES RICH HAMARTOMA OF GILLS IN NON-DISEASED ATLANTIC SALMON (*SALMO SALAR* L.)

M.N. Yousaf[1]*, A.B. Amin[2]

[1]*Norwegian Veterinary institute, Harstad, Norway*
[2]*Patologikonsult AS*

Gills are the main respiratory organ which serves for gaseous exchange, acid base regulation and excretion in fish. Salmonids gills typically comprise two sets of four paired cartilaginous gill arches placed on either side of the head. Each gill arch is supporting a holobranch with its two hemibranchs, the double vertical rows of gill filaments. Hemibranchs consist of a row of long thin filaments and the surface area of which is increased by the formation of semilunar folds across its dorsal and ventral surface termed as lamellae. The gill tissues are in contact with surrounding environment (water) and are vulnerable to infectious and non-infectious diseases. Hamartoma defines as "*a focal malformation of tissue that resembles a neoplasm, grossly and even microscopically, but results from faulty development in an organ*". Pulmonary hamartoma are common tumours in humans as compared to its equivalent gill tumours are rare in teleost fish. Here we present a case report of an eosinophilic granulocytes rich mesenchymal hamartoma in the gills of Atlantic salmon. The tumour was identified in the gills of an otherwise non-diseased fish. The round to oval mass of 4,5 cm x 2,5 cm x 1,5 cm was present on the posterior end of lamellae. Histological examination showed hamartoma of normally presented cartilage tissue, fibrosis, spindle shaped cells, eosinophilic granulocytes, epithelial hyperplasia/hypertrophy, mucous hyperplasia, focal bleedings, cavern formation and pseudo epithelium. In addition mono-nuclear lymphocytes infiltration and necrosis/degeneration were also identified. Histologically, there was focal mitotic activity close to outer border which may also contribute in the tumuor development. Immunohistochemistry using proliferating cell nuclear antigen (PCNA) identified cell proliferation. To the best of our knowledge, histological features of such tumours in salmonids have not been previously described and extended the list of tumours reported in fish.

STUDY OF SKELETAL ANOMALIES IN SENEGALESE SOLE (*SOLEA SENEGALENSIS*) FED WITH DIETS USING DIFFERENT COMMERCIAL ENRICHMENT PRODUCTS

A.M. DE AZEVEDO[1], A.P. LOSADA[1], P. RONZA[1], I. FERREIRO[2], A. RIAZA[2], L. PRESTINICOLA[3], C. BOGLIONE[3], S. VÁZQUEZ[1], M.I. QUIROGA[1]*

[1]*University of Santiago de Compostela, Lugo, Spain*
[2]*Stolt Sea Farm, A Coruña, Spain*
[3]*University of Rome "Tor Vergata", Rome, Italy*

The production of a promising flatfish species, as Senegalese sole, is affected by a high incidence of vertebral deformities noticed in up to 40% of the juveniles. Investigation in this subject has related the development of skeletal anomalies with culture conditions and nutritional causes. The imbalance of some nutrients in the diet can originate alterations in bone physiology, provoking deformities. Few studies exist about the influence of enrichment products on the development of skeletal alterations. The purpose of this study was to evaluate the presence of vertebral anomalies in soles fed with diets enriched with different commercial products. Metanauplii of *Artemia* were enriched with four distinct products and used to feed Senegalese sole larvae. Individuals were reared in two separated tanks for each diet and sampled at 31 and 105 days after hatching (DAH). Double staining technique with Alcian Blue and Alizarin Red was performed. Specimens were evaluated for the detection of anomalies in parapophysis, neural and haemal processes, vertebral bodies and caudal complex elements. Statistical study involved NPMANOVA tests and Correspondence Analysis. The percentage of individuals that presented skeletal anomalies was very high, reaching 100% and 99.5% at 31 and 105 DAH, respectively. Alterations like deformation of the caudal haemal arches/spines, fusion and deformation of hypurals, epural or parhypural were common to all the lots. Fusions and/or alterations of the shape of preurals were the most common anomalies concerning vertebral bodies. At 31 DAH, the Correspondence Analysis output ordered fish from each diet in separated quadrants, but at 105 DAH, soles from diets B and C located together in the fourth, thus revealing a shared pattern of anomalies. Therefore, despite some patterns of anomalies were perceived for each diet, they were not maintained over time. Further research is required on additional rearing conditions effects and the development of skeletal anomalies in this species.
Supported by “Consellería de Economía e Industria”, Xunta de Galicia (10MMA020E), Spain. AM de Azevedo holds a University Professorship Formation (FPU) grant from the Spanish Ministry of Education.

NUTRACEUTICS FOR THE CONTROL OF BACTERIAL COLDWATER DISEASE

C. FERNÁNDEZ-ÁLVAREZ[1], Y. TORRES-CORRAL[1], G. COSCELLI[3], A. R. SÁNCHEZ-ARÉVALO[4], A. MARTÍNEZ[4], M.I. QUIROGA[2], Y. SANTOS[1]*

[1]*Facultad de Biología (CIBUS)*
[2]*Facultad de Veterinaria, Universidad de Santiago de Compostela, Spain*
[3]*Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Argentina*
[4]*Lípidos Toledo S.A, Madrid Spain*

The aquaculture industry has developed significantly over recent decades and is, today, one of the sectors of the world's fastest growing food production. One of the most important problems that affect aquaculture occurs in parallel to its growth, and is the appearance of disease in fish. Among the most important diseases caused by bacteria in aquaculture, Bacterial Cold-Water Disease caused by *Flavobacterium psychrophilum*, produces high mortality and morbidity in cultured salmonid fish worldwide. Evidence indicating that antibiotic-resistant bacteria and antibiotic resistance determinants pass from the aquatic to the terrestrial environment has resulted in a drastic restriction of the use of antibiotics in aquaculture in many countries. For this reason, it is important to find new products with antimicrobial activity without the aforementioned undesirable effects of antibiotics. In this sense, natural food additives or phytobiotics, which combined different mechanisms of action against pathogenic bacterial species (bactericidal/bacteriostatic activities, Quorum Sensing inhibition...), are potential candidates for the development of prevention strategies in aquaculture.
The present work described the effect of the dietary administration to rainbow trout of the phytobiotic Liptofry (Liptosa S.A, Spain) in the improvement of disease resistance against *Flavobacterium psychrophilum*. Information about survival and pathological findings observed in control and treated fish after experimental infection trial will be discussed.

This work was supported by Grant IDI-20140147, Centro para el Desarrollo Tecnológico e Industrial (CDTI), Ministerio de Economía y Competitividad, Spain.

INTESTINAL MICROBIOTA OF ATLANTIC SALMON (*SALMO SALAR* L.)

J. LOKESH*, H.Q. DANG, V. KIRON

Faculty of Biosciences and Aquaculture, University of Nordland, Bodø, Norway

Profiling the host-associated microbiota forms the first step towards understanding the symbiotic relationship between the host and the microbes. The present study deals with the taxonomic characterization of microbiota of the distal intestine of Atlantic salmon using 16S rRNA gene (V3 region) sequencing. Bacterial communities connected with the fish during different developmental stages and in adults were studied. At the first feeding stage, the diversity of the intestinal community increased significantly, and eventually during the freshwater phase of the fish a stable population, evenly represented by Firmicutes and Proteobacteria, was established. The seawater transfer phase was characterized by a significant change in the community composition with Proteobacteria becoming overabundant. Proteobacteria was identified as the most dominant phylum in adult fish collected from different locations. Phylum-level compositional difference between the microbiotas of these fish was not detected. The findings indicate that the temporal variation in the community structure is caused by external stimuli such as feeding and rearing environment, whereas a spatial variation in the community is not evident at the phylum level.

WATER AS A SOURCE OF MACRO- AND MICROELEMENTS IN FISH NUTRITION

J. PAJDAK[1]*, E. TERECH-MAJEWSKA[1], P. SCHULZ[1], W. SZWEDA[1], A.K. SIWICKI[2]

[1]*University of Warmia and Mazury, Olsztyn, Poland*
[2]*Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland*

Macro- and microelements are needed in small quantitives but play a key role in many metabolic processes. Trace mineral and macromineral deficiencies may go unnoticed due to an absence of clear clinical symptoms in fish. Deficiencies of these elements can lead to impaired health in fish, reduced body weight gain and lower feed conversion.
In contrast to terrestrial animals fish can ingest minerals from food or directly from water. Absorption processes are determined by various factors, mostly mineral concentrations in water but also other water parameters.
The required dietary supplementation of macro- and micronutrients is very difficult to determine, and the amount of nutrients absorbed by fish from water is equally difficult to measure. The interactions between these elements should also be taken under consideration. Many authors emphasize that the phosphates may reduce the absorption of most micronutrients. Also, the current parameters of the water can affect the bioavailability, e.g. phosphorus absorption increases with increasing temperature.
Some elements (e.g. calcium) can be absorbed from the ambient water in the quantity sufficient to meet the demand for this element. Other elements, however, require supplementation in the diet, for eg. studies indicate the need for supplementation of phosphorus. It is worth to note that in the case of some macro- and micronutrients, excessive Mount is much more important than their deficiency is excessive amount in the water. An example might be zinc, the excess of which can be toxic.
Measurement of water parameters such as temperature, pH, nitrate and nitrite levels and the amount of dissolved oxygen are a regular feature of environmental control in fish farming. Determination of macro and micro levels, however, is still not common in aquaculture. Measurement of these parameters could suggest which elements need to be supplemented and which are found in water in an amount that meets the needs of the fish.

EFFECT OF *ORIGANUM VULGARE* L. ON THE GROWTH PERFORMANCE AND ANTIOXIDANT STATUS OF RAINBOW TROUT (*ONCORHYNCUS MYKISS*)

Ö. DILER*, Ö. GÖRMEZ, İ. DILER, S. METIN

[1]*Department of Aquaculture, Faculty of Egirdir Fisheries, Suleyman Demirel University, Isparta, Turkey*

The research was aimed to determine the effect of different increasing levels of *Origanum vulgare L.* essential oil as feed additives in rainbow trout diets in order to observe their influence on growth performance, antioxidant activity and some histological alternations in liver and kidney. Fish (26-27 g) were fed the experimental diets for 90 days. Experimental diets supplemented with four different concentrations (0.125, 1.5, 2.5, 3.0 ml/kg). Fish fed diets containing essential oil of *O. vulgare L.* had significantly higher final weight and growth than the control group except 0.125 in *O. vulgare L.*. Feed conversion ratio in fish fed diets containing 1.5 and 3.0 ml kg^{-1} essential oil of *O. vulgare L.* was improved than other treatments ($p<0.05$). Antioxidant status of fish was assayed for levels of plasma superoxide dismutase (SOD), plasma catalase (CAT) and lysozyme activity. Results showed that the levels of plasma superoxide dismutase activity and lysozyme activity were higher in *O. vulgare L.* group compared to control. This results suggested that the essential oil of *O. vulgare L.* can be applied as growth promoter, increase antioxidant activity when added to rainbow trout feed.

REMINERALISATION OF THE PRIMARY PHOSPHORUS-DEFICIENT SKELETAL PHENOTYPE IN POST SMOLT ATLANTIC SALMON (*SALMO SALAR*)

P.E. WITTEN[1], M.A.G. OWEN[2]*, M. SOENENS[1], C. MCGURK[2], A. OBACH[2]

[1]*Ghent University, Department of Biology, Ledeganckstraat 35, 9000 Ghent, Belgium*
[2]*Skretting Aquaculture Research Centre, P.O. Box 48, N-4001, Stavanger Norway*

We have previously described a phosphorus (P) deficient vertebral phenotype in post-smolt juvenile *Salmo salar* (Witten et al. 2015). The animals have P deficient vertebral bodies that are un-deformed with a large amount of peripheral un-mineralised normal bone matrix surrounding a core of regular mineralised bone.
The current study examined if animals displaying this mineral deficient vertebral phenotype were able to remineralise the un-mineralised bone matrix.
Nine tanks of 30 salmon were fed either a low P or requirement P diets for eight weeks. After which five animals per tank were randomly sampled for radiographic and chemical analysis. Animals fed the LP diet displayed the characteristic P-deficient vertebral phenotype. RP fed animals had a normal vertebral conformation and no apparent deformity. At this point the six tanks fed LP diets were randomly assigned to one of three diets; a continuation on the low phosphorus diet (LP, N=2), or a change to high phosphorus or requirement phosphorus (RP) treatments (N=2). The animals fed the RP treatment for the initial portion of the experiment continued on this diet (N=3). All tanks were fed for a further ten weeks after which ten animals per tank were radiographed and whole body and opercula samples taken.
The deficient vertebrae remineralised when fed either the RP or HP diets without apparent vertebral malformation. In addition the whole body and opercula ash content was commensurate in animals fed the RP and HP diets following P restriction. Animals that continued on the LP diet were not deformed but displayed the characteristic phosphorus deficient vertebral phenotype.
These results confirm that the P deficient vertebral phenotype of post smolt salmon is not deformed and the vertebrae can remineralise following a period of P-restriction.

EFFECT OF EYE FLUKES (TREMATODA, DIPLOSTOMATIDAE) UPON ACTIVITY OF GUT DIGESTIVE ENZYMES IN BAIKAL OMUL (*COREGONUS MIGRATORIUS* GEORGI, 1775)

M.M. Solovyev[1]*, O.T. Rysinek[2]

[1]*Institute of Animal Systematics and Ecology, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*
[2]*Baikal Museum, Siberian Branch, Russian Academy of Sciences, Listvyanka, Irkutsk oblast, Russia*

The ability to digest consumed food in fish depends on the presence of digestive enzymes. These enzymes are characterized by certain localization in the gut wall and along the digestive tract cavity. As a rule the levels of activity of these enzymes along the fish intestine vary depending on the feeding habits and morphology of the intestine (Ugolev and Kuz'mina, 1993; Deguara et al., 2003).
Infection with parasites may be one of the factors influencing the activity of digestive enzymes. The reports on the influence of parasites on the activity of digestive enzymes are very scarce and contradictory. It was reported that the activities of amylase, protease, and acid phosphatase in mucosa and in the gut content of common carp yearlings infected with *Bothriocephalus acheilognathi* were decreased (Davydov and Kurovskaya, 1991; Kurovskaya, 1991). Infection with parasites may limit the ability of hosts to consume food resulting in changes in the catalytic capability of intestinal enzymes (Kurovskaya, 1991).
Specimens of Baikal omul (*Coregonus migratorius*) not infected or infected with trematoda Diplostomum sp. served as study objects. The fish were caught in August 2013 in Lake Baikal. The lengths of the studied fish (measured according to Smith) varied from 40 to 50 mm. The fish guts were removed and frozen immediately in liquid nitrogen until analysis. The number of the eye flukes was counted in each eye. The activity of total alkaline proteases, trypsin, chymotrypsin, alkaline phosphatase, aminopeptidase, and maltase were determined as recommended by Gisbert et al., (2009). The activity of amylase and nonspecific lipase was detected as recommended by Deguara et al., (2003) and Gawlicka et al., (2000) respectively. In order to assess statistically significant effects the correlation analysis was used (Statistica 6.0).
The significant influence ($r=-0.3–0.4$; $p\leq0.05$) was found between activity of brush border enzymes (alkaline phosphatase, aminopeptidase and maltase) and numbers of eye flukes. For activity of pancreatic enzymes the significant effect of the number of eye flukes was not detected. It could be demonstrated that the number of eye flukes influence on both basic type of process in intestine as digestion and absorption.

The study was supported by the Russian Foundation for Basic Research, project no. 13-04-00270 A.

MOLECULAR CHARACTERIZATION AND EVOLUTION OF ITALIAN VHSV AND IHNV

M. ABBADI*, V. PANZARIN, A. FUSARO, R. QUARTESAN, M. TOSON, L. BILLE, M. DALLA POZZA, A. TOFFAN, G. CATTOLI

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

Viral haemorrhagic septicaemia virus (VHSv) and infectious hematopoietic necrosis virus (IHNv), belonging to the *Rhabdoviridae* family, are the most important and severe viral pathogens for rainbow trout farming. In Italy, despite the entry into force of the most recent European directive 88/2006 and the application of eradication programs, several trout farms are still being affected by these two viruses, leading to great losses in economic terms. The objective of this study was to investigate the epidemiology and the evolution of VHSv and IHNv in northern Italy in the past 20 years. For this purpose, we have selected representative viral isolates deriving from rainbow trout sampled during several outbreaks which had occurred in different fish farms in the time period between 1982-2013. A total number of 108 VHSv and 89 IHNv samples were phylogenetically analysed by sequencing the complete G-gene ORF. For both datasets, the rates of nucleotide substitutions/site/year (sub/site/y) and the time of the most recent common ancestor (tMRCA) were also estimated.

The phylogenetic analysis revealed that the Italian VHSv strains belong to the genotype Ia, sublineages Ia1 and Ia2, and are distributed within 7 different genetic clusters, suggesting the occurrence of different viral introductions in Italy over time. Oppositely, all IHNv isolates were typed as belonging to the E (European) genogroup, although it was not possible to identify different genetic clusters as for VHSv. The estimation of the evolutionary rate shows that IHNv has evolved more rapidly than VHSv (1.1×10^{-3} sub/site/y *vs* 7.4×10^{-4} sub/site/y). The tMRCA calculated for both VHSv and IHNv was consistent with the first report of these pathogens in Italy. Finally, sequence data and related epidemiological information were combined to better understand diffusive dynamics of VHSv and IHNv in our country. Different scenarios for viral spread among different trout farms were hypothesized.

Our analyses have shed light on VHSv and IHNv epidemiology in northern Italy, highlighting the importance of combining bioinformatic approaches with epidemiological data to track the viral flow and plan adequate surveillance strategies in acquaculture.

WGS-AQUA: CAPACITY BUILDING FOR THE WIDESPREAD ADOPTION OF WHOLE GENOME SEQUENCING (WGS) FOR THE MOLECULAR EPIDEMIOLOGY OF AQUACULTURE PATHOGENS

S.C. BAYLISS[1]*, E.J. FEIL[1], A. ADAMS[2], D.W. VERNER–JEFFREYS[3], D. AANENSEN[4], R. VAN AERLE[3], C. BAKER-AUSTIN[3], K.L. BARTIE[2], O. BRYNILSRUD[5], D. COLQUOUN[6], B. MABLE[7], J. MARTINEZ-URTAZA[1], T.P.H. NGO[2], S. RUSSELL[9], K.M.E. TURNER[10], S. SHEPPARD[11], N.C. STENSETH[5], D. STONE[3], T. WALLIS[12]

[1] *University of Bath, Bath, UK*
[2]*University of Stirling, Stirling, UK*
[3]*Cefas Weymouth Laboratory, Weymouth, UK*
[4] *Wellcome Trust Genome Campus, Cambridge, UK*
[5]*Norwegian University of Life Sciences, Oslo, Norway*
[6]*Norwegian Veterinary Institute, Oslo, Norway*
[7] *University of Glasgow, Glasgow, UK*
[9]*Elanco Animal Health Canada, Victoria, Canada*
[10]*University of Bristol, Bristol, UK*
[11]*University of Swansea, Swansea, UK*
[12]*Ridgeway Biologicals, Compton, UK*

The advent of next-generation whole genome sequencing (WGS) of pathogens has greatly enriched our understanding of the evolutionary and ecological processes underpinning disease emergence and spread. Whilst most of the current focus is on terrestrial pathogens of public health importance, many of the opportunities afforded by WGS are transferable to aquaculture settings. The adoption of WGS for targeted studies and, ultimately, routine surveillance of aquaculture pathogens, would represent a turning point in confronting the significant challenges associated with sustainable aquaculture.

The over-arching aim of the BBSRC/NERC-funded project WGS-AQUA is to facilitate the widespread adoption of WGS for aquaculture disease management. This is achieved by bringing together UK and Norwegian bioinformaticians, modellers, statisticians and population genomicists working at the forefront of infectious disease epidemiology in the public health arena, with key stakeholders and academics in the aquaculture sector. The aim is to optimise and implement a community-oriented WGS database infrastructure with associated software tools designed for 'top down' visualisation and analysis of pathogen spread (wgs-aqua.net). The broad utility of WGS, and of the software, will be demonstrated by preliminary results on genome sequences of multiple key aquaculture pathogens.

AQUAPONICS: KEY ELEMENTS AND CONSIDERATIONS

D. JAKIĆ-DIMIĆ[1]*, V. RADOSAVLJEVIĆ[1], Z. MARKOVIĆ[2], D. LJUBOJEVIĆ[3], T. LABUS[4], M. ĆIRKOVIĆ[3]

[1]*Institute of Veterinary Medicine of Serbia, Belgrade, Serbia*
[2]*Faculty of Agriculture, Belgrade, Serbia*
[3]*Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia*
[4]*Ministry of Agriculture and Environmental Protection, Belgrade, Serbia*

Aquaponic join recirculating aquaculture with hydroponics to use nutrient waste from aquaculture as an input to plant growth. In aquaponics, the waste products from the fish are converted by a bio-filter into soluble nutrients which are absorbed by the plants, and allow "clean" water to be returned back to the fish. Thus, it produces valuable fish protein with a minimal pollution of fresh water resources, while at the same time producing horticultural crops. Plants are grown separately in hydroponic tanks, submerged in water but suspended in gravel, sand, perlite, or porous plastic films, as well as on floating rafts. Systems vary greatly in design and construction, but most perform the following key functions: finfish and plant production, removal of suspended solids, and bacterial nitrification. Aquaponic systems are usually designed as an enclosed recirculating system, but a few systems can be open, depending upon environmental factors. Fish or other aquatic organisms are reared in tanks and excrete nutrient-rich waste or effluents into the water. Metabolic byproducts excreted by fish, unionized ammonia, ionized ammonia, or combined equal total ammonia nitrogen are oxidized and broken down into nitrite by nitrifying bacteria of the genera Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio. There are multiple aquaponic system designs that have been analyzed and utilized for crop production. The characteristics of recirculating systems make aquaponics less prone to pathogen introductions and disease outbreaks because of better control of inputs and in the management of key water and environmental parameters. Most of the mortalities in aquaponics are not caused by pathogens, but rather by abiotic causes mainly related to water quality or toxicity. Nevertheless, such agents can induce opportunistic infections that can easily occur in unhealthy or stressed fish. This paper discusses fish health problems, effects and perspective of aquaponics.

FIELD VALIDATION OF A MODEL FOR RISK BASED SURVEILLANCE OF AQUACULTURE IN SWITZERLAND

N. DISERENS[1]*, L. FALZON[2], B. VON SIEBENTHAL[1], T. WAHLI[1]

[1]*Centre for Fish and Wildlife Health, Bern, Switzerland*
[2]*Veterinary Public Health Institute, Bern, Switzerland*

To guarantee equivalence to the council directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals, Switzerland needs to establish a risk-based surveillance of aquaculture facilities. The Centre for Fish and Wildlife Health (FIWI) therefore developed in a first project a model for risk-based surveillance of fish farms regarding the viral hemorrhagic septicemia (VHS) and the infectious hematopoietic necrosis (IHN). The model included 6 factors to assess the risk of an introduction of VHS and IHN into a fish farm, as well as 7 factors to assess the risk of the spreading of the two diseases from an aquaculture facility. The data used to feed the model were collected by means of a questionnaire that was sent to all known Swiss aquaculture facilities. Based on the model calculations, the farms were then classified into different risk categories. These risk categories form the basis to determine the control frequencies for effective disease surveillance in aquaculture facilities.

However, several factors (e.g. factors regarding biosecurity) could not be collected by means of the questionnaire. Moreover, the accuracy of the data provided by the fish farmers is uncertain. In the here presented project the data collected with the questionnaire were verified and completed, by means of aquaculture inspections in 4 selected pilot cantons (Bern, Vaud, Valais and Zurich).

For several factors a poor agreement between data gathered with the questionnaire and those derived from inspections was found while other factors were in perfect agreement. Thus actualisation and complementation of the original data with the field data resulted in a considerable change in the risk ranking for some of the aquaculture facilities. Some of the farms were visited twice within 6 months which allowed to assess potential changes within this period. In some of these farms clear differences in some factors were evident.

With this project, the challenges of the application of a risk based method for fish disease surveillance are highlighted and particularly the advantages and disadvantages of the different approaches to get the necessary data for performing a risk ranking are depicted.

WITHDRAWN P-105

THE IMPACT OF MARINE SAV2 INFECTION ON PRODUCTION PERFORMANCE IN NORWEGIAN SALMONID AQUACULTURE

M.D. JANSEN*, D. JIMENEZ, H. VILJUGREIN

Norwegian Veterinary Institute, Oslo, Norway

Pancreas disease (PD) continues to be a major challenge for Norwegian salmonid aquaculture. Following the introduction of a new subtype of Salmonid alphavirus (SAV) to mid-Norway in 2010, the number of PD outbreaks caused by marine SAV2 has increased sharply. As marine SAV2 established itself, a widespread interest emerged in determining subtype-differences in clinical manifestation and production performance. In line with field observations, recent epidemiological investigations have found PD outbreaks due to marine SAV2 to result in lower mortality and milder clinical signs compared to outbreaks due to SAV3. Despite mild clinical manifestations, field observations indicate that infection with marine SAV2 results in reduced production performance compared to uninfected cohorts. So far, no scientific quantification and evaluation of the impact of marine SAV2 in Norwegian aquaculture has been performed.
This study compares production performance of marine SAV2-infected cohorts to that of non-infected cohorts within the SAV2-zone. The following parameters will be evaluated: mortality level, growth rate, biological feed conversion rate, fish quality grading at slaughter and consumer complaints on fillet quality. The results will provide significant knowledge regarding the impact of marine SAV2, thereby aiding informed recommendations regarding possible mitigation- and management measures within the SAV2-zone.

RESISTANCE TO ANTIPARASITIC DRUGS IN THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*)

P.A. JANSEN[1]*, R. GRØNTVEDT[1], A. TARPAI[1], K.O. HELGESEN[2], T.E. HORSBERG[2]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*Norwegian University of Life Sciences, School of Veterinary Science, Oslo, Norway*

The Norwegian Veterinary Institute is responsible for carrying out a surveillance and control program focusing on resistance in salmon lice to different antiparasitic drugs. The aim of the program is to provide an overview of the spatio-temporal distribution of drug efficacy along the Norwegian coast, as a basis for evaluating salmon lice control strategies. The present paper analyses results from bioassay-testing of salmon lice from salmon farms along the Norwegian coast, using the drugs azamethiphos and deltamethrine. Lice were exposed to either high or low concentrations of either drug for 24 hours, and the outcome of the test was whether lice immobilized or not[1]. The data were analyzed using logistic mixed modeling with farm as the random variable. Altogether 60 farms were tested with bioassays in 2013 and 79 farms in 2014. We found that the predicted probability of lice dying in the bioassay tests depended on the local density of drug treatments. Furthermore, lice were generally highly susceptible to the drugs in the far north, yielding high lice-mortality in the tests from these areas. The lowest sensitivity with low mortality predictions was found in Mid Norway, whereas areas in the south showed intermediate sensitivity. Results from 2014 yielded considerably lower model predictions for lice mortality than for 2013. This was partly due to a year effect in the mixed model, but partly also due higher local treatment densities in 2014. The random effects attributed to farms were rather large, indicating large pre-test uncertainties in the outcome of tests for given farms. We conclude that reduced sensitivity to antiparasitic drugs in the salmon louse is widespread along the Norwegian coast. The association between local treatment densities and predicted lice mortality in the bioassay tests probably reflect a causal relationship between treatment and development of resistance.

[1]*K.O. Helgesen & T.E. Horsberg 2013. Single-dose field bioassay for sensitivity testing in sea lice, Lepeophtheirus salmonis: development of a rapid diagnostic tool. J. Fish Dis. 36: 3 261-272.*

ECOANTIBIO: IMPROVE THE MONITORING SYSTEM FOR ANTIMICROBIAL USE AND ANTIBIOTIC RESISTANCE

S. LE BOUQUIN[1]*, S. BARON[1], A. TOCQUEVILLE[2], M. GAUMÉ[2], J. DAVID[1], M. JAMIN[3], I. KEMPF[1], C. CHAUVIN[1]

[1] *ANSES, French Agency for Food, Environmental and Occupational Health and Safety, Ploufragan, France*
[2] *ITAVI, Rouen, France*
[3] *FILI@VET- Saint Martin des Champs, France*

Antimicrobial resistance is acknowledged by health organizations as one of the major health challenges of the XXIst century. To address this problem, the EU authorities have implemented action plans, both in human and animal health.
In France, the "Ecoantibio" program has been designed to reduce the use of veterinary drugs by 25% in 5 years. To allow assessing the impact of the plan and the completion of its objective it is necessary to establish the baseline at the beginning of the program. Few data are available on the fish farming industry in France, but they show a low exposure to antimicrobials compared to other intensive livestock sectors. Considered as a minor production in France, the therapeutic arsenal available is reduced. The efforts conducted the last 15 years to reduce antimicrobial use have been connected with the development of vaccination.
In this context, ANSES has developed several research projects in coordination with the professionals. These projects are based on a global approach of the farms, from the environment to the fish, and integrate microbiological data on pathogenic, commensal and/or indicator bacteria, antibiotic use and health practices.
The AnPi study started in 2014. This transversal study was designed to get an overview of use of antibiotics in freshwater salmon farming and establish a baseline. A representative sample of a hundred farms spread all over the French territory was investigated, using an epidemiological questionnaire. Data on the characteristics of the farm, the water environment, sanitary practices, diseases and antimicrobial use were collected. Currently underway, this study is expected to provide information on the quantities used by therapeutic class, on terms of use and on associated factors.
In parallel, a pilot study, AquaRes, was conducted to assess the presence of resistant bacteria (*Aeromonas*, *Enterococcus*) in the water, upstream and downstream of 20 farms. The objective of this study was to constitute a collection of environmental isolates.
Finally, in 2015, the FlorCo project has been initiated to assess the presence of resistant bacteria (*Aeromonas*, *Enterococcus*, Vibrio, *Plesiomonas*,...) within the commensal flora of trout collected in 20 farms.

THE MOST FREQUENT AND THE MOST IMPORTANT DISEASES IN SALMONID BREEDINGS IN CONDITIONS OF THE CZECH REPUBLIC

Z. MARKOVÁ*, S. NAVRÁTIL, I. PAPEŽÍKOVÁ, M. PALÍKOVÁ

University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

Breeds of salmonid fish in the Czech Republic are endangered by both obligatory and facultative pathogens. The list of these diseases was assembled on the basis of long-term monitorg of the health status of fish. The outbreak of disease is ussually a consequence of the breach of optimal conditions for breeding.
Viral diseases from the list of danger diseases are very serious problem in the aquaculture. In the Czech Republic is very important viral haemorhagic septicaemia, because 13 focuses of this disease were detected in the year 2014. Number of the focuses of infectious haematopoietic necrosis was lower.
The biggest problems in salmonid breads in the Czech Republic are bacterial pathogens. The most important disease is furunculosis caused by *Aeromonas salmonicida* subsp. *salmonicida*, enteric red mouth disease caused by *Yersinia ruckeri* and cold-water diseases caused by *Flavobacterium psychrophilum.* These diseases are very frequently accompanied with secondary fungal cotton wool disease.
The conditions of the aquaculture are suitable for relative low number of parasitic species, but their influence is much stronger than in open waters. The serious problem in our breads is white spot disease caused by *Ichthyophthirius multifiliis*, ichthyobodosis caused by *Ichthyobodo necator*, proliferative kidney disease caused by *Tetracapsuloides bryosalmonae*, gyrodactylosis caused by *Gyrodactylus truttae* and nematodosis caused by *Raphidascaris acus.*
The defficiency of oxygen and high concentration of nitrogen compouds (ammonia, nitrites) can cause serious problems in recirculating systems, especially after damage of biofilters.
Salmonid breads in the Czech Republic are endangered with a lot of pathogens. Their infuence can be eliminated by suitable zoohygienic conditions, good nutrition, conforming to the veterinary rules, disruption of developmental cycles of parasites and alternatively by suitable treatment.

The study was supported by project NAZV (QJ 1210013) and by IGA VFU Brno (246/2015/FVHE).

FISH HEALTH IN SERBIA DURING THE PERIOD 2009-2014

V. RADOSAVLJEVIC[1]*, D. JAKIC-DIMIC[1], V. MILICEVIC[1], D.LJUBOJEVIC[2], M. CIRKOVIC[2]

[1]*Institute of Veterinary Medicine of Serbia, Belgrade, Serbia*
[2]*Scientific Veterinary Institute, Novi Sad, Serbia*

Fish farming industry in Serbia is mainly based on cyprinid species common carp (*Cyprinus carpio*) and salmonid species rainbow trout *(Oncorhynchus mykiss)*. In addition, silver carp (*Hypophthalmichthys molitrix),* bighead carp (*Hypophthalmichthys nobilis*), grass carp (*Ctenopharyngodon idellus*), wels (*Silurus glanis*), pike-perch (*Stizostedion lucioperca*), northern pike (*Esox lucius*) and sterlet (*Acipenser ruthenus*) are cultured. Total fish production in Serbia varied from 12 to 14 thousand tonnes per year. Due to the increasement of disease problems in the fish farming industry and the impact disease may have on both feral and farmed fish, monitoring and surveillance on diseases have for many years been considered to be of great importance. Serbia has a surveillance programme for Viral Haemorrhagic Septicaemia (VHS), Infectious Haematopoietic Necrosis (IHN) and Koi Herpes Virus (KHV) based on EU regulations and a monitoring programme for Infectious Pancreatic Necrosis (IPN), Spring Viremia of Carp (SVC) and Bacterial Kidney Disease (BKD) on a national level. For the bacterial and parasitic diseases, standard diagnostic procedures have been used for screening purposes. All types of farms are included in the survey such as hatcheries, brood stock farms as well as grow out farms. During five-year period, presence of fish diseases were examined on 56 carp ponds and 52 trout ponds. IPN is considered to be the main viral disease problem, encountered every year in trout farms and number of positive farms is rising. Sleeping disease in rainbow trout is detected first time in 2014. Red mark sindrom is present since 2009, occuring regularly. Also, ranaviral infection caused by European catfish virus (ECV) in brown bullhead (*Ameiurus nebulosus*) was detected every year during previous period. Last case of SVC was detected in 2009. Also, KHV was not detected during this period. Infections caused by Flavobacteria, *Yersinia ruckeri*, *Renibacterium salmoninarum*, motile aeromonads and *A. salmonicida* occurred most frequently. Also, *Philometroides cyprini* infection in silver crucian carp and Eustrongilus infection in *Sander lucioperca* were detected in 2014.

VIRAL HAEMORHAGIC SEPTICAEMIA (VHS) AND *INFECTIOUS HAEMATOPOIETIC NECROSIS* (IHN) HEALTH STATUS IN SLOVENE AQUACULTURE

M. SENIČAR*, D. ŽELE, R. SITAR, V. JENČIČ

National Veterinary Institute, Veterinary Faculty University of Ljubljana, Slovenia

Situated in Alpine and Prealpine area Slovenia has great salmonid aquaculture potential. Salmonids for the fishing waters repopulation as well as rainbow trout for the consumption purpose is bred. In last two decades the number of salmonid fish farms has increased. Although we've got many water sources, they are not huge and therefor the capacity of the majority of farms is low. In Slovenia great attention has always been paid on the fish diseases prevention and the national surveillance programme framed in the Annual Decree is continually performed by the veterinarians of the National Veterinary Institute. Entering the EU the Directive 2006/88/EC was implemented as well as the list of the registered and authorised trout aquaculture businesses was established and is available on the website of the Administration of the Republic of Slovenia for Food Safety. All fish farms are categorised in five health statuses and regularly visited regarding the diseases risk levels. The majority of the fish farms are categorised in the third health category meaning that they are under veterinary control but not yet included in the program to obtain the diseases free status or they are not interested in it at all. We are encouraging fish farmers to enter the program as for the end of year 2018 the VHS/IHN approved health status will be mandatory for all fish farms producing fish for introduction and repopulation. The most important viral health problem is IHN, which was diagnosed for the first time in 1996 and is currently spread. Currently there are 28 IHN positive fish farms Slovenia. In these farms the disease occasionally still occurs while new outbreaks are rare. In 2008 also VHS was diagnosed in several fish farms; however with the proper veterinary measures its further spreading was prevented. At the moment there are 7 VHS positive fish farms where eradication program has not been carried out officially. Recently, no new VHS outbreaks has been *detected*.
In the poster salmonid aquaculture and the epizootiological situation regarding VHS and IHN will be presented.

RISK RANKING OF IRISH SALMON FARMS BASED ON NETWORK METRICS AND BIOSECURITY EVALUATION

T. YATABE[1]*, S.J. MORE[2], F. GEOGHEGAN[3], C. MCMANUS[4], A.J. HILL[1], B. MARTÍNEZ-LÓPEZ[1]

[1]*University of California, Davis, USA*
[2]*University College Dublin, Dublin, Ireland*
[3]*Marine Institute, Rinville, Ireland*
[4]*Marine Harvest Ireland, Rinmore, Ireland*

Live fish movement has been deemed as having an important role in the transmission of infectious diseases locally and globally. The characterization of the patterns and dynamics of live fish movements in a region or country provides not only a better understanding of how fish diseases can spread but also how to allocate interventions for more cost effective disease prevention and control programs. Biosecurity is another important factor in determining farm vulnerability for disease incursion and spread. Effective biosecurity strategies provide protection to both farmed and wild aquatic animal populations, by minimizing the risk of introducing pathogens and minimizing the consequences or further spread if a pathogen was introduced.

Here we analyzed the structure and dynamics of live Atlantic salmon, *Salmo salar*, movements in Ireland during the years 2009 through 2014 using social network analysis methods, and evaluated the biosecurity practices at the farm level through the application of a detailed survey to site managers, which was used to generate a farm biosecurity score. Finally, these results were used as an input for elaborating a risk ranking, to identify sites at higher risk of disease introduction and/or spread, in order to allocate surveillance resources more efficiently, within the framework of risk-based surveillance.

Resulting site centrality measures from the network analysis (indegree, outdegree, betweenness, incloseness, and outcloseness) and biosecurity scores were analyzed using Principal Component Analysis (PCA) to reduce the number of dimensions in the data into a set of variables explaining most of the observed variability. With the resulting principal components, a K-means clustering algorithm, which partitions a dataset into k distinct non-overlapping clusters while minimizing the within-cluster variation, was carried out, setting k = 3 groups aiming to produce 3 risk categories: low, medium and high.

It is important to note, that besides live fish movement and biosecurity within the industry, there are possibly other factors involved in a site's susceptibility to disease. Hence this risk ranking is meant as a preliminary step to direct further research on the pathways or routes that contribute the most to disease occurrence on a farm.

STUDY ON MULLET FISH STOCKS REDUCTION AND PRESENTATION OF CONTOL AND PREVENTION METHODS OF VIRAL NERVOUS NECROSIS (VNN) DISEASE IN THE CASPIAN SEA

M.E.J. ZORRIEHZAHRA[1]*, M. GHASEMI[2], M. GHIASI[3], S.H. KARISIDANI[4], G. BOVO[5], A. NAZARI[6], H.H.M. DAUD[7], M. ADEL[3], S.T. DELSHAD[8]

[1] *Iranian Fisheries Research Organization, (IFRO), Tehran, I.R. Iran*
[2] *Inland Water Aquaculture Research Center, Bandar Anzali, I.R. Iran*
[3] *Mazandaran Fisheries Research Center, Sari, Iran*
[4] *Islamic Azad University, Bandar Anzali Branch, Bandar Anzali, I.R.Iran*
[5] *Instituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy*
[6] *Islamic Azad University, Falavarjan Branch, Isfahan, Iran*
[7] *Faculty of Veterinary Medicine, University of Putra Malaysia (UPM), Malaysia*
[8] *School of Veterinary Medicine, Shiraz University, Shiraz, I.R. Iran*

Catching of Mullet fishes have been varied in recent years and was increased from 2500 MT in 1991 to 6700 MT in 2002 but some reduction was observed such as 2700 MT in 2010 and 2151 MT in 2012. Mullet fishes always feeding from the bottom sea as detritus habits and have important role in the filtration of nutrients in the bottom Sea. The feeding habits of this species include benthic organisms and detritus, making it particularly adapted to accumulate sediment-associated contaminants and thus an important bio-indicator species. Unknown mortality has been occurred in wild mullet in the Caspian Sea since 2003. According to primary study and pilot plan that Viral Nervous Necrosis was confirmed by OIE Reference Laboratory in Italy and Japan and concerning to comprehensive research project it could be concluded that VNNV was the main causative agent of recent outbreaks in Mullet of Caspian Sea. In continuous investigation that was done on affected Mullet with pathognomonic clinical signs such as abdominal distention and belly up at rest through Virology assay (Cell culture), confirmation methods (IFAT & IHC), histopathology and hematology studies similar findings were obtained in winter season of 2011 and 2012. These findings have been indicated continuous persistence of VNN in Caspian Sea. Regarding to variation of aquatic biodiversity in the Caspian Sea and susceptibility and sensitivity of some economic fish to VNN virus, focus on increment of susceptible hosts in marine and fresh water fish that passed from 57 species in the world, and more tendency to Cage Culture and other intensive culture methods, more investigations on VNNV were carried out in recent years. Most of them were focused on Rapid diagnosis methods, suggestion of decrement ways for virus loading in water environments, identification of intermediate hosts, production of fry and juvenile free of virus, introduce of Immune System upgrading methods such as using of immunostimulators products, produce of effective vaccines with high coefficient of protection, identification of virus life cycle and introduce of cutting ways in the nature and presentation of applied protocols for control and prevention. So, most important methods of control, prevention and health management requirements would be discussed in order to decrement of economic loses and spread of disease in region.

COMPARATIVE HISTOPATHOLOGY OF HERPESVIRUS INFECTION IN *SCOPHTHALMUS MAXIMUS* AND *SOLEA SENEGALENSIS*

M. CONSTENLA[1]*, A. RIAZA[2], R. SILVA[2], B. ALONSO[2], F. PADRÓS[1]

[1]*Universitat Autònoma de Barcelona, Cerdanyola del Vallés, E-08193 Barcelona, Spain*
[2]*Stolt Sea Farm, 15292 A Coruña, Spain*

Infections with herpesviruses are common in fish being probably the Cyprinid herpesvirus (CyHV) one of the most important groups. Some years ago, *Herpesvirus scophthalmi* infection in turbot *Scophthalmus maximus* was described but since then very few other descriptions on this particular condition has been published. However, routine health controls indicate that infections associated to herpesvirus are not uncommon in turbot and are also occasionally detected in Senegalese sole *Solea senegalensis*. I both cases, the disease is mainly noticed by behavioural changes and mucus hypersecretion. Histologically, in turbot the typical large giant cell were observed in gill and skin epithelium, usually singly, situated both in the base of the lamellae and in the apical portion thereof. These cells usually have a rounded central nucleus more basophilic than the cytoplasm. In addition to these cells, inflammatory foci, mainly composed by macrophages, were observed especially in chronic infections. In sole, only gills seem to be infected and two types of giant cells were observed: ones resembling *H. scophthalmi type*; and another ones smaller, which consists of a granular basophilic material with a hypertrophic nucleus especially large. The latter were observed in much greater numbers than in turbot.. These lesions are usually accompanied by mild to moderate inflammatory necrotic lesions, often associated with the presence of proteinaceous exudate and clusters of lymphocytes in the subepithelial space. Potentially associable to this lesional gill pattern, clusters of inflammatory cells clearly distinguishable from normal parenchyma and surrounded by proteinaceous exudate were often observed in other organs such as kidney and spleen, which could correspond with vascular lesions induced by inflammatory herpesvirus cells. In addition, in both fish species, mild to moderate inflammatory lesions were often observed in skin, usually localized in the epidermis and dermis. These lesions were superficial and also associated to a vascular pattern; however, hypertrophic cells have never been observed in the skin. Transmission electronic microscopy has been performed in sole in order to verify the viral nature of the lesions and large numbers of polyhedral or round capsids with a core were detected.

DETECTION OF *NUCLEOSPORA* SPP. IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FROM CAGE FISH FARMING ON HYDROELETRIC RESERVOIRS IN BRAZIL

C.J. FRANCISCO*, M.V. RODRIGUES, J.P. ARAÚJO JÚNIOR, M.C.C. DE SOUZA, M. BONGIOVANI, F. HENRIQUE, G.S. DAVID, R.J. DA SILVA

Univ. Estadual Paulista (UNESP), Distrito de Rubião Júnior, São Paulo, Brazil

Nucleospora spp. is an intra-nuclear microsporidian parasite of the family Enterocytozoonidae. Until now these parasites were reported infecting salmonid fishes: *Oncorhynchus mykiss*, *O. aquabonita*, *O. kisutch*, *Salmo salar*, *Salvelinus namaycush*, and *S. fontinalis*. These parasites were also reported in non-salmonids fish as *Cyclopterus lumpus*, *Nothobranius rubripinnis* (aquarium fish), *Hippoglossus hippoglossus*, and *Parophrys vetulus*. The main target cell for this parasite is hematopoietic cells, which undergo proliferative changes leading to a leukemia-like condition with an accompanying anemia. This research aimed to report *Nucleospora* spp. with molecular techniques from kidney of Nile tilapia (*Oreochromis niloticus*) sampled in Brazil. It was sampled 30 tilapia of each fish farming (n=6). A nested-PCR was performed with the specific primers of 16S srRNA region for *Nucleospora* for all samples. The amplified products were purified and sequenced by Sanger method. Of 180 fish analyzed, 68 (37.77%) was positive by nested-PCR, wherein fish farming 1 had 5 positives animals (16.66%), fish farming 2 had 10 positives animals (33.33%), fish farming 3 had 7 positives animals (23.33%), fish farming 4 had 19 positives animals (63.33%), fish farming 5 had 8 positives animals (26.66%), and fish farming 6 has 19 positive animals (63.33%). As described by others authors, the target organ was kidney and the prevalence by PCR could vary since 10% to 100%, as observed in this study. Since necropsy and histopathology changes can only suggest that the infection could be caused by anmicrosporidian, thereby molecular techniques can confirm the pathogen suspected. Once, observation could spend a long time, PCR assay is faster, cheaper, sensitive and specific test for diagnosis propose. This is the first report of *Nucleospora* spp. infecting kidney of Nile tilapia.

OUTBREAK OF CRYPTOSPORIDIOSIS IN SEA CAGED TURBOT (*PSETTA MAXIMA*)

M.L. FIORAVANTI[1]*, F. QUAGLIO[2], M. CAFFARA[1], A. GUSTINELLI[1], O. PALENZUELA[3], S. KRIZANAC[4], S. ČOLAK[4]

[1]*Department of Veterinary Medical Science, Alma Mater Studiorum University of Bologna, Italy*
[2]*Department of Comparative Biomedicine and Food Science, University of Padova, Italy*
[3]*Instituto de Acuicultura Torre de la Sal, IATS-CSIC, Castellón, Spain*
[4]*Cromaris, Croatia*

Turbot (*Psetta maxima*) is currently one of the most promising species for European mariculture but although most of the critical aspects threatening production have been optimized, transmissible diseases may still represent a limiting factor. In autumn 2014 two batches of 5 turbot (mean weight 305g) caged in northern Adriatic Sea and showing melanosis, exophthalmos and skin/fin lesions were subjected to bacteriological and parasitological examination. In addition, visceral organs of twenty fish from the same two cages were fixed directly on the field in 10% buffered formalin. Necropsy showed in all specimens pale-yellow liver and intestinal distension with abundant mucous contents. From the fish owing to one batch ascites was also observed and *Vibrio scophthalmi* was isolated at the bacteriological exam. Routine parasitological examination did not reveal parasites in any organs except for the intestines, where developmental stages of unidentified parasites were observed. In order to identify the parasite, portions of intestine from all fish were subjected to DNA extraction followed by amplification of 18S rDNA portions. BLAST search of the sequences permitted to ascribe the parasites to the genus *Cryptosporidium*. In the histopathological study, a mild-to-severe infection by cryptosporidia was detected in the intestine of all fish from both cages. Epicytoplasmic stages of parasites lining the luminal surface of the epithelial cells were observed, generally with the presence of intra-epithelial sporogonic stages located deeply within the enterocytes layer. In some areas, oocysts were present within vacuolated areas in the mucosa, with diffuse necrosis, sloughing off the epithelium and loss of the enterocytes brush border; *lamina propria* and submucosa were oedematous. Inflammatory response involving mainly lymphocytes and, in heavy infections, rodlet cells was noticed. In some fish also the stomach was affected. Severe pathological changes were mainly observed in relation to the presence of numerous intra-epithelial oocysts. The histopathological findings here described are similar to those already reported in farmed turbot in Spain, due to *Cryptosporidium scophthalmi*. In our case the source of infection could not be assessed since fish had been introduced into the cages from abroad several months before. The role of wild carrier fish cannot be ruled out.

NODULAR GILL DISEASE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*): EMERGENCE IN ITALY

M. GALEOTTI[1]*, D. VOLPATTI[1], C. BULFON[2], P. BERALDO[1], P. PERANTONI[3], G. MATTIUZZI[3], G. ROSSI[2], G. MAGI[2]

[1]*Department of Food Science, Veterinary Pathology Section, University of Udine, Italy*
[2]*School of Bioscience and Veterinary Medicine, University of Camerino, Italy*
[3]*Veronesi Holding, Aqua Division, Verona, Italy*

Nodular gill disease (NGD) affecting rainbow trout (*O. mykiss*), firstly described by Daoust and Ferguson (1985) in USA, has been subsequently reported in Denmark and Poland. The present outbreaks have been surveyed in rainbow trout farmed in 6 fish farms in north Italy, during the spring season (T=10°C). Symptomatic fish showed hyperventilation, anoxia, gills hyperplasia and loss of reactivity. Mortality reached 60-70%. Macroscopically the gills showed multi-focal pearly white nodular proliferation of lamellae and increased mucus secretion. Lesions were often bilateral. Fish sizes ranged from 100 to 150 g. Forty-six symptomatic fish were sampled and euthanized for the investigation. Samples of skin/muscle, gills, liver, heart, spleen, kidney, were fixed in Formalin and Bouin; gills samples were fixed also in 2.5% glutaraldehyde for TEM. The histological evaluation highlighted a massive epithelial proliferation that often lead to a complete obliteration of the affected arches. Very frequently, we detected lamellar fusion affecting the entire filament or its distal portion. In the gills epithelium numerous cells, possibly ascribable to amebae, were detectable, showing a large central nucleus containing a thin peripheral layer of chromatin, which was separated by a clear halo from the nucleoplasm. They were adherent or included in the epithelium. Mitotic figures were common and cellular debris were present in the external part of the hyperplastic lamellae. TEM analysis revealed few trophozoites. They were irregularly round (8-12 µm), showing a slight electron-dense filamentous coat and an abundant cytoplasm containing numerous mitochondria, evident endoplasmic reticulum, scattered free ribosomes and few clear small vacuoles. Most of the mitochondria had lamellar cristae, only few of them had discoid shape. These findings may suggest to ascribe them to *Naegleria* spp., as described by Dykova and Kostka (2013). This is the first report concerning NGD outbreaks in rainbow trout farmed in Italy.

COMPLETE GENOME SEQUENCE OF EELPOUT RHABDOVIRUS (ERV) IDENTIFIED BY DEEP SEQUENCING OF VIRAL RNA

M. HAKHVERDYAN[1], M. ARESKOG[1]*, E. BLOMKVIST[1], A. ALFJORDEN[1], T.S. BOUTRUP[2], H. AHOLA[1], F. LJUNGHAGER[3], Å. HAGSTRÖM[1], N.J. OLESEN[2], M. JUREMALM[1], J.-F. VALARCHER[1], C. AXÉN[1], M. LEIJON[1]

[1]*National Veterinary Institute, Uppsala, Sweden*
[2]*Technical University of Denmark, Copenhagen, Denmark*
[3]*Swedish Agency for Marine and Water Management, Gothenburg, Sweden*

By deep sequencing of RNA we identified the genome of a previously undescribed rhabdovirus causing mass mortalities in Baltic Sea eelpout (*Zoarces viviparous*) in 2014.The virus was subsequently named eelpout rhabdovirus (ERV). Eight virus positive samples were used; four samples from pooled tissue and cell culture supernatants originating from this pool (Outbreak#1, May), and four cell culture supernatants from four different eelpouts (Outbreak#2, October).
Following RNA extraction, cDNA tag-labelling, random amplification, primer sequence removal and final product purification, libraries were normalized by adjusting concentration to 2 nM with nuclease-free water, pooled and further prepared for sequencing on the MiSeq platform. All experiments were analyzed using CLC Genomics Workbench 7.5.2. Contigs where blasted against a locally created virus BLAST database. A maximum likelihood phylogenetic tree was created by alignment with a selection of rhabdovirus genomes.
On average the eight samples produced 4.8 million paired-end reads, and after de novo assembly, approximately 129,000 contigs each. BLAST hits were dominated by rhabdoviruses, the majority were Siniperca Chuatsi rhabdovirus (SCRV) and hybrid snakehead virus. Consensus sequences were extracted using the longest contigs. Coverage ranged from ca 1,200-52,000 reads. A consensus sequence of 11,139 nucleotides was obtained by aligning the Outbreak#2 sample sequences. When aligned with the genome of its closest relative (SCRV), the sequence displayed an overall identity of 59.5% at overlapping positions, identifying it as a new virus. Open reading frames (ORFs) with starting codons exactly aligning with those of SCRV were found for all typical rhabdovirus proteins except for the glycoprotein, that was shifted four amino acids away. No ORF corresponding to M_s was found. Since Outbreak#1 samples originated from one organ pool, a single de novo assembly was used and a final consensus sequence of 8,837 nucleotides was obtained in the same way as for the Outbreak#2 samples. Pair-wise comparison of Outbreak#1 and Outbreak#2 sequences revealed an identity of 99.92-99.98 % for sequences within Outbreak#2 and 99.76-99.77 % between Outbreak#1 and Outbreak#2 sequences. Lack of variability between viral RNA from Outbreak#1 and Outbreak#2, which were both geographically and temporally separated, shows that ERV is an emerging virus in the Baltic Sea.

CLEANER FISH: NEW SPECIES UNDER THE MICROSCOPE

M. MARCOS-LÓPEZ[1]*, M. ALARCÓN[2], H. HELLBERG[4], M. MCLOUGHLIN[3], M. PEARSON[3], T. TURNBULL[3], K.I. LIE[4], H.D. RODGER[1]

[1]*Vet-Aqua International, Oranmore, Ireland*
[2]*Norwegian Veterinary Institute, Harstad, Norway*
[3]*Fish Vet Group Scotland, Inverness, UK*
[4]*Fish Vet Group Norway, Oslo, Norway*

Sea lice is one of the most important health challenges for the farmed Atlantic salmon *Salmo salar* industry worldwide. Sea lice populations can develop resistance to the medical treatments applied. In order to overcome this and on work towards a more sustainable industry, cleaner fish are successfully used as biological control for sea lice. The use of cleaner fish is growing rapidly and is now a general practice of many farms in Scotland and Norway, and in increasing demand in Ireland. The fish species used are mostly ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*) and lumpsucker (*Cyclopterus lumpus*). As new aquaculture species, there is a need for increasing our knowledge on diseases affecting these species (both infectious and non-infectious). The intensification and potential stressful conditions of their rearing is likely to increase the health challenges for these species. This poster identifies and illustrates the most important diseases in these species known to date, including, among others, vibriosis, atypical furunculosis, *Pasteurella* sp., amoebic gill disease and *Nucleospora cyclopteri infection.*

CARP EDEMA VIRUS (CEV): FIRST DETECTION IN ITALY

T. PRETTO*, M. ABBADI, V. PANZARIN, R. QUARTESAN, A. MANFRIN, A. TOFFAN

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

Carp Edema Virus (CEV) is an unclassified virus belonging to the *Poxviridae* family, which was first detected and described in Japan in the '70s. It is associated with severe edema of juvenile common carp, resulting in a high mortality rate. More recently, the same virus has also been associated with lethargic behaviour in adult koi carp. Since then, this pathologic condition has been defined as "Koi Sleepy Disease" (KSD). KSD has been reported in Europe for the first time in 2009, although a retrospective study dates the first introduction of CEV in Europe back to the '80s. Pathogenesis, epidemiology, prevalence and impact of CEV for European aquaculture are still unknown.

In Italy, the first confirmed CEV outbreak was detected in May 2014 from diseased 1-2 kg common carp (*Cyprinus carpio*) imported from Hungary. Fish showed lethargy, sleepy behaviour, marked hydropsy and acute mortality shortly after arrival, with water temperature of 23°C. At necropsy, fish presented cutaneous mucus hyperproduction on the flank region and the caudal fin. Gills were pale with abundant mucus and necrosis of the tip of the gill filaments was observed. Internal organs appeared enlarged (trunk kidney and spleen) and the edematous intestinal tract showed seromucosal content. *Aeromonas sobria* and *Streptococcus sp*. were isolated from spleen and kidney. Nested PCR (protocol provided by CEFAS, unpublished) performed on gills and trunk kidney samples yielded positive results for CEV.

A backward-reconstruction of CEV introduction in Italy was perfomed by analysing carp organs stored in our repository. Our survey confirmed that the first occurrence of CEV in Italy dated back to 2010, when a severe outbreak involving common carp from a fishing pond was recorded, with high mortality affecting more than 600 fish in few weeks at low water temperature (6-7°C). Nested PCR successfully detected CEV in 3 year-old common carps sampled during this outbreak. Histological examination of the gill tissues showed epithelial hyperplasia leading to extensive lamellar fusion with moderate necrosis and mild lymphocitic infiltration, while trunk kidney and encephalon showed congestion.

Co-infection with CyHV-1, *A. sobria* and *A. hydrophila* was also detected.

FIRST DESCRIPTIONS OF NODULAR GILL DISEASE IN ITALIAN FARMED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

F. QUAGLIO[1]*, A. PEROLO[1], P. BRONZATTI[2], A. GUSTINELLI[3], V. MENCONI[3], G. CAVAZZA[3], M. CAFFARA[3], A. MANFRIN[4], E. GALLO [1], M.L. FIORAVANTI[3]

[1]*University of Padova, Italy*
[2]*Veterinarian fish pathologist*
[3]*Alma Mater Studiorum University of Bologna, Italy*
[4]*Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy*

Amoebic gill disease is a severe parasitic condition affecting salmonid farming in marine and freshwater environment. Several outbreaks of nodular gill disease associated with amoebic infections in freshwater farms of rainbow trout (*Oncorhynchus mykiss*) have been reported in Denmark, Poland, Germany, USA and Canada. Some cases of nodular gill disease in juvenile and adult rainbow trout have been observed during the last three years in some trout farms in northern and central Italy. High mortality rates occurred throughout the year, with a higher severity and incidence at low temperatures. Clinical signs were indicative of respiratory distress. Gross pathology observations of gills showed excessive mucus production, whitish nodules in the distal regions of filaments and alternated anemic and congested areas. The nodules commonly were composed of several adjacent filaments clubbed and fused together, suggesting diffuse and severe epithelial hyperplasia. The lesions observed were evaluated utilizing a categorical field evaluation of gross gill score. In some samples examined in spring 2014 the microscopic exam of fresh gill showed the presence of roundish elements of about 20 µm in diameter referable to amoebae lined up along the gill surface or piled between the secondary lamellae. The identity of amoebae has not been determined so far and is still under study. Histological examination of gills revealed extensive proliferation of the epithelial and mucous cells, often with complete fusion of the lamellae. Lamellar oedema and leucocyte infiltration in the hyperplastic tissue were found. The filaments in severe cases were totally coalescing, sometimes affecting the entire gill arch. The apical portion of the filament showed marked spongiosis accompanied by cellular sloughing. Amoebae, better evidenced by Giemsa staining, were observed adhering to the gill epithelium surface and within interlamellar spaces. Bacteriological exams of the gills did not support a correlation between presence of bacteria and amoebic infections. Nodular gill disease of trout is an emerging problem which requires particular attention by fish pathologists. Studies are in progress to clarify etiopathogenesis and main environmental and biological determinants of the disease in order to define appropriate measures for its prevention and control.

CHROMOSOME-ENCODED ENZIMATIC ACTIVITIES AS CANDIDATE VIRULENCE FACTORS OF *PHOTOBACTERIUM DAMSELAE* SUBSP. *DAMSELAE*

A. VENCES*, M. TERCETI, A.J. RIVAS, M. BALADO, C.R. OSORIO

Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Photobacterium damselae subsp. *damselae* causes fatal septicemia in fish and other marine animals, and also in humans. A fraction of strains, which mainly include turbot and human isolates harbour the 153 kb virulence plasmid pPHDD1, that encodes two hemolysins: the phospholipase Damselysin (Dly) and the pore-forming toxin $HlyA_{pl}$, the two constituting virulence factors for fish. In addition, all the hemolytic isolates regardless of their isolation source produce a chromosomally-encoded ($HlyA_{ch}$) toxin, with a suspected minor contribution to virulence. Thus, the two main virulence factors described to date are plasmid-encoded and are restricted to a fraction of the isolates. Indeed, most isolates do not contain pPHDD1, albeit they are toxic for fish and cell lines. Therefore, in the present study we aimed at identifying candidate virulence factors in plasmidless strains following two approaches: on the one side, an *in silico* search in complete genome sequences revealed candidate genes encoding potential toxins. On the other side, by using transposon mutagenesis we isolated mutants deficient in specific enzymatic activities. By combining the two approaches we have identified a number of chromosome-encoded candidate genes: (i) a collagenase that is restricted to a small fraction of the isolates, (ii) a phospholipase (Plp_{ch}) that proved to be ubiquitous in the subspecies and distinct from the pPHDD1-encoded Dly phospholipase, and (iii) an ubiquitous lipase/esterase activity with its associated fatty acid transporter. Moreover, we have demonstrated that a type-two secretion system (T2SS) participates in the secretion of the collagenase, whereas T2SS mutants were not altered in production of lipases and Plp_{ch}. Fish toxicity assays using extracellular products from strains containing different combinations of deletions in the candidate genes are under way. The ubiquitous lipase/esterase and phospholipase activities constitute firm candidates to play a role in toxicity and virulence. The identification of virulence markers that explain the pathogenicity of *P. damselae* subsp. *damselae* is essential for future strategies against vibriosis caused by this emerging pathogen.

DETECTION OF CARP EDEMA VIRUS IN COMMON CARP (*CYPRINUS CARPIO*) AND KOI CARP IN THE CZECH REPUBLIC

T. VESELÝ*[1], D. POKOROVÁ[1], S. RESCHOVÁ[1], V. PIAČKOVÁ[2]

[1]*Veterinary Research Institute, Brno, Czech Republic*
[2]*University of South Bohemia in České Budějovice, Czech Republic*

Koi sleepy disease (KSD) was first mentioned in Japan in the second half of the 1970s. Prior to dying, the affected fish were lying on the bottom, further, the gills were damaged and bodies became swollen. Electron microscopy of ultrathin sections showed coated virus particles morphologically corresponding to poxviruses. The causative agent of the disease started to be named "carp edema virus" (CEV).
Some cases, which were clinically similar to koi herpesvirus disease (KHVD), appeared in the Czech Republic in 2013 and 2014, however, the virus was not confirmed. Carp samples showing gill necrosis, skin lesions or koi sleepy disease signs were therefore examined using modified nested PCR assay for CEV, which was developed at CEFAS UK.
CEV was confirmed in a carp from a locality with fish dying and in a koi carp with sleepy disease signs in a hobby garden pond. Both the findings were sequenced and it was found that sequence section of 505 nucleotides from koi carp was identical with the data from Japan, whilst the sequence from common carp was 95.8% identical.
This is the first detection of carp edema virus in the Czech Republic.

This study was supported by Ministry of Agriculture of the Czech Republic projects QJ1210237 and MZE 0002716202.

LASER DISSECTION MICROSCOPY AND DNA SEQUENCING FACILITATES IDENTIFICATION OF NOVEL EPITHELIOCYSTIS ASSOCIATED BACTERIA

J. WIIK-NIELSEN[1]*, H.T. SOLHEIM[1], T.M. STEINUM[2], G. BORNO[1], H.R. SKJELSTAD[1], A.B. OLSEN[1], A.G. GJEVRE[1], D.J. COLQUHOUN[1]

[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*University of Istanbul, Istanbul, Turkey*

Epitheliocystis, an infection characterised by cytoplasmic bacterial inclusions (cysts) in the gill and less commonly skin epithelial cells, has been reported in many marine and freshwater fish species and may at times be associated with pathological changes and mortality. Bacteria associated with epitheliocystis have not yet been successfully cultured. The number of bacterial species described and associated with this condition is, however, growing slowly, due largely to retrieval of genetic information by molecular biological means. Until recently, only bacteria related to the *Chlamydiae* were associated with this type of infection. This made selective retrieval of 16S rRNA sequence information possible using '*Chlamydiae*-specific' PCR primers. Recently, however, two non-Chlamydial taxa i.e. Ca. *Branchiomonas cysticola* and *Endozoicomonas elysicola* have been identified as agents of epitheliocystis, suggesting that chlamydia based sequence retrieval may be too narrow for use in future studies. 'Universal' bacterial primers are, however, likely to amplify any bacterial sequence within or associated with the gill tissues including both surface associated and ubiquitous aquatic taxa.
As part of a project aimed at mapping the epitheliocystis-related bacterial taxa present in Norwegian salmon farming we developed a system involving visualisation of epitheliocysts in non-stained paraffin embedded tissue sections by fluorescence *in situ* hybridisation (FISH). Visualised epitheliocysts were then dissected using laser dissection microscopy, total DNA extracted and analysed either by specific qPCR or by PCR amplification using 'universal' primers amplifying stretches of the 16S rRNA gene followed by DNA sequencing. The method was used to screen a number of archived paraffin embedded blocks collected from a wide geographical area covering a period of 10 years. The method was successful in retrieving relatively pure nucleic acids from the bacteria under investigation in quantities suitable for PCR and subsequent sequencing. The survey identified both previously known and novel agents of epitheliocystis in Norwegian farmed Atlantic salmon.

COCKLE *CERASTODERMA EDULE* MARTEILIOSIS FIRST DETECTED IN RÍA DE AROUSA (GALICIA, NW SPAIN) HAS SPREAD TO OTHER GALICIAN RÍAS CAUSING MASS MORTALITY

D. IGLESIAS[1], A. VILLALBA[1], E. NO[1], S. DARRIBA[2], C. MARIÑO[3], J. FERNÁNDEZ[4], M.J. CARBALLAL[1]*

[1]*Centro de Investigacións Mariñas (CIMA), Consellería do Medio Rural e do Mar, Xunta de Galícia, Vilanova de Arousa, Spain*
[2]*Instituto Tecnolóxico para o Control do Medio Mariño de Galicia (INTECMAR), Consellería do Medio Rural e do Mar, Vilagarcía de Arousa, Spain*
[3]*Cofradía de Pescadores "San Antonio" de Cambados, Cambados, Spain*
[4]*Xefatura Comarcal de Vilagarcía de Arousa, Consellería do Medio Rural e do Mar, Xunta de Galícia, Vilagarcía de Arousa, Spain*

The cockle *Cerastoderma edule* fishery has traditionally been the most important shellfishery in Galicia in terms of biomass. Mass cockle mortality affecting most cockle beds of the Ría de Arousa was recorded in 2012, due to infection with the protistan parasite *Marteilia cochillia*, which had never been detected previously in the region. Marteiliosis was highly prevalent and affected both juvenile and adult cockles causing the cockle fishery collapse in this ría and a huge decrease in cockle Galician landings. The high pathogenicity of *M. cochillia* presaged an uncertain future for the affected beds and led to consider marteiliosis a serious threat to the whole Galician cockle fishery. In order to detect the presence of *M. cochillia* in new locations, a surveillance programme begun in December 2012, involving bimonthly sampling in the rías adjacent to Ría de Arousa and less frequently in other Galician cockle beds. Marteiliosis dynamics was analysed by sampling monthly two affected beds: *Lombos do Ulla* and *Sarrido*, located in the inner and outer zone of the Ría de Arousa, respectively. *M. cochillia* was detected causing mortality in cockle beds of Ría de Pontevedra, in 2013, and Ría de Vigo, in 2014, thus confirming disease spreading to southern rías. Every new cockle recruitment wave in ría de Arousa has been affected by marteiliosis since 2012, avoiding fishery recovery. Marteiliosis outbreaks showed different temporal pattern in *Lombos do Ulla* and in *Sarrido*, suggesting an influence of the estuarine gradient on the disease dynamics. *M. cochillia* was first detected in *Lombos do Ulla* and the infection progressed very quickly causing 100% mortality in 2-3 months, while first detection in *Sarrido* delayed 2-3 months and infection progression was slower, but also causing mass mortality. Thus, the bad presages have been confirmed. Effort should be focused on avoiding further *M. cochillia* spreading.

PAPILLARY LESIONS IN THE MEDITERRANEAN MUSSEL *MYTILUS GALLOPROVINCIALIS* FROM ITALIAN AND SPANISH COASTS

F. CARELLA[1]*, N. CARRASCO[2], D. FURONES[2], G. DE VICO[1]

[1]*Department of Biology, University of Naples Federico II, 80134 Napoli, Italy*
[2]*IRTA, Sant Carles de la Ràpita, Ctra. Poblenou Km 5, 43540, Tarragona, Spain*

Several descriptions of neoplasms have been reported in marine bivalves so far. Some of them, like gonadal and haemic neoplasia, are the most common and share the characteristic of malignant tumours. Other types, less frequent, are the tumours arise from epithelia, muscle and connective tissue and usually classified as benign since neither invasive behaviour nor mitotic figures are usually observed. Among them, papillomas are benign epithelial tumours morphologically characterized by cauliflower-like branched projections pedunculated or sessile. In higher vertebrates and in different animal species, they are frequently associated to viral agents. In marine bivalves, such structures of epithelial origin have been observed in oysters like *Saccostrea commercialis* and *Ostrea virginica*. No reports are currently available in literature concerning the occurrence of papillomas in the mediterranean mussel *M. galloprovincialis*, and the description in mytilidae family is represented by abnormal growth in mantle cells of *Modiolus difficilis* in Japan Sea. In this paper we describe for the first time naturally occurring cases of papillomatosis in mediterranean mussels in two coastal areas of the Mediterranean Sea, Spain (NE) and Italy (SW). In May 2011, 7 animals over 30 (23%) in Spain and July 2013 4 over 20 (20%) in Italy showed papillary lesions arising from the mantle and hearth epithelium. At the heart, such growth were multiple and constituted by finger-like papillary projections, showing columnar and cuboidal cells connected by a fibro-vascular core, as underlined by the trichrome stain. In the mantle epithelium, they were sessile and distributed in different areas. The growths showed a pluristratified hyperplastic epithelium constituted by swollen and irregularly shaped cells. Interestingly, the lesions were always associated to an inflammatory component in some case atypical, showing PCNA positivity in two individuals and presence of nuclear inclusion-*like* bodies. The hysto-pathogenetic results are discussed.

MORPHOMETRIC STUDY OF NORMAL, INFLAMMATORY AND NEOPLASTIC HAEMOCYTES IN MEDITERRANEAN MUSSEL *MYTILUS GALLOPROVINCIALIS*

F. CARELLA[1]*, G. DE VICO[1], G. LANDINI[2]

[1]*Department of Biology, University of Naples Federico II, 80134 Naples, Italy*
[2]*School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*

Nuclear morphology is a central feature associated with cellular function and disease. Enlargement of nuclei, anisokaryosis, nuclear pleomorphism, changes in chromatin patterns and nucleolar abnormalities are well-recognized features of neoplastic cells. The quantitative assessment of variability of such nuclear features in pathological and physiological conditions has important diagnostic applications that can be best achieved using morphometric analysis.
Disseminated neoplasia (DN) is a proliferative disorder of bivalve haemocytes firstly described by Farley (1969) in oysters (*Crassostrea virginica* and *Crassostrea gigas*). It was later observed in twenty other molluscan species from various ecosystems and geographic areas, including commercially-important species, and reported in populations suffering mass mortality. Previous studies on bivalve species revealed abnormal DNA content in normal and neoplastic haemocytes. Despite that nuclear morphometry and DNA densitometry have been extensively studied in human neoplasia, there have been few applications in the field of comparative pathology. The aim of this work was to quantify the nuclear morphometry and DNA contents of neoplastic cells of mussels *M. galloprovincialis* affected by DN using Feulgen stained histological preparations. The obtained data were also compared with a population of normal and inflammatory haemocytes in mussels from the Campania region with different degrees of inflammation.
We captured 256 images of 3 different DN stages, 120 images of normal and 124 of inflammatory haemocytes, totalling 120,166 cells to be analysed. We extracted 21 morphological parameters from normal and neoplastic haemocytes (expressed as: *normal vs neoplastic* cells) among which nuclear Area (224,4 ±94,1 *vs* 423,1±226,9), Perimeter (58,3 ±14,0 *vs* 79,0±21,3) and Integrated Optical Density (70,5 ±34,5 *vs* 177,1±150,8), were found to be relevant features that allowed discrimination between normal, inflammatory and neoplastic haemocytes. Stepwise Linear Discriminant Analysis according to 5 distinguished classes showed 68,3% of cases were correctly classified, also showing that at stage 1 the 55,8% of the cells could classified as stage 2. The presence of two distinctive population of neoplastic cells with different IOD, Area and Perimeter were identified. Computer-based imaging is one of such methods that allows the direct visualization of the cell populations, providing simultaneous correlation between ploidy measurements and morphological features.

DIGESTIVE EPITHELIAL VIROSIS (DEV)–LIKE AFFECTION IN MEDITERRANEAN COMMERCIAL BIVALVES

N. CARRASCO[1]*, F. CARELLA[2], I. GAIRÍN[1], G. DE VICO[2], M.D. FURONES[1]

[1]*IRTA, Sant Carles de la Ràpita, Ctra. Poblenou Km 5, 43540, Tarragona, Spain*
[2]*Department of Biology, University of Naples Federico II, 80134 Napoli, Italy*

The Digestive Epithelial Virosis (DEV)– like pathological signs have been described since 1979 in different bivalve species including mussels, scallops and cockles (*Mytilus edulis*, *Patinopecten yessoensis*, *Pecten alba*, *Pecten maximus* and *Cerastoderma edule*) in diverse world locations such as New Zealand, Australia, Japan, Great Brittany, Denmark, Scotland and Spain. Most of those species are relevant commercial bivalves. However little information on the non-identified aetiological agent implicated and the disease progressis currently available. Furthermore, the impact of such pathology is still poorly known. Presence of DEV-like tissue alterations reaching prevalence up to 100% has been recently reported on European common cockle *C. edule* from Mediterranean waters. Such observation pointed out the importance of increasing epidemiological knowledge on such pathological condition in Mediterranean molluscs. Pathology of commercial bivalve species, with emphasis on DEV, was thus studied in three different coastal areas of the Mediterranean Sea, two in Spain (NE and SE) and one in Italy (SW), in samples recollected between the years 2007 and 2014. DEV-like tissue alterations were observed in non-previously described species such as the variegated clam *Chlamys varia* and the Manila clam *Ruditapes philippinarum*, besides *C. edule*, reachingprevalences up to 93.34%. Ultrastructural and histopathological observation allowed the identification of the presence of different phases of the disease progression, which were described in detail, in a try of understanding the developmental sequence of the disease and the levels of severity. Thus, this is the first study focusing on the different pathological aspects of DEV-like pathology including epidemiology, disease development and evolution, tissue tropism and host damage from a comparative approach including different bivalve affected species from European waters.

COMPARISON OF DIFFERENT FIXATIVES AND FIXATION METHODS FOR LIGHT MICROSCOPIC EXAMINATION OF THE GASTRO-INTESTINAL TRACT OF PACIFIC WHITE SHRIMP (*PENAEUS VANNAMEI*)

F. CERVELLIONE[1]*, C. MCGURK[1], W. VAN DEN BROECK[2], P.F. SILVA[1]

[1]*Skretting Aquaculture Research Centre, Stavanger, Norway*
[2]*Ghent University, Ghent, Belgium*

Health assessment of aquatic species is fundamental to apply new prophylactic and therapeutic strategies to support and enlarge the aquaculture sector and consequently the availability of high valuable animal proteins for human population. Penaeid shrimp aquaculture is an important global industry, with Pacific White Shrimp, *Penaeus vannamei*, one of the most cultured species so far. Many diseases target the gastro-intestinal tract of crustaceans, including the hepatopancreas. Histological examination and image analysis represent powerful potential tools to assess the health status and the morphological changes in the organs and tissues.
Hepatopancreas undergoes rapid autolysis immediately after death and delays of even a few seconds of fixative penetration into this organ can result in the whole specimen being unsuitable for diagnostic assessment.
The aim of this study was to optimise the fixation protocol for shrimp tissue, focusing on the gastro-intestinal tract, including the hepatopancreas, to achieve high quality tissue sections, which can be used for image analysis.
Animals were weighed and divided into groups, from 0.8 grams to 6.0 grams. Five fixatives (Davidson´s, Bouin´s, Carnoy´s, Neutral buffered formalin, Zinc salts-based fixative) and two fixation methods (immersion versus injection) were tested. Five different volumes (6%, 10%, 20%, 30%, 40% of total body weight) were injected into animals in intermoult stage C. Three different injection protocols (single injection into the hepatopancreas, multiple injections into the hepatopancreas, and multiples injections around the hepatopancreas) were compared. After fixation for 48-72 hours, tissues were processed and embedded in paraffin. Subsequently, 8µm thick sections were made and stained with haematoxylin and eosin. Using light microscopy, a 0-5 semi-quantitative scale was established to evaluate the fixation rate of the hepatopancreas.
Data showed a clear correlation between volumes injected, injection points, type of fixative and fixation achieved.

BIVALVE HAEMOCYTES: A MODEL SYSTEM FOR PCNA DYNAMICS IN HUMAN INFLAMMATORY GRANULOCYTES AND LYMPHOMAS

G. DE VICO*, A. COSTANTINO, F. CARELLA

Department of Biology, University of Naples Federico II, Naples, Italy

PCNA, the auxiliary protein of DNA polymerase-δ and ε, is a highly phylogenetically conserved protein, usually localized in the nucleus, where it is involved in cell proliferation control and DNA repair. Recent studies in human neutrophils suggest PCNA could also relocalize in the cytoplasm where it is involved in sustaining inflammation by suppressing granulocytes apoptosis, a key stage in the resolution of inflammatory conditions. Cytoplasmic PCNA also occurs in human lymphoma cells, but its role remains to be elucidated. We present here the evidence that the same PCNA distribution pattern (nuclear/cytoplasmic) observed in human granulocytes and lymphomas is detected in normal, inflammatory and neoplastic bivalve haemocytes, suggesting PCNA dynamics occur in a similar manner in both human and bivalve immune cells. According to Wessler (1976) animal models are living organisms "...in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respect resembles the same phenomenon in humans or other species of animal". As a consequence, bivalve haemocytes could represent an emerging and unique animal model for studying PCNA dynamics in human neutrophils and leukemia. This could be relevant for discovery new therapeutic approaches in both human inflammatory conditions and lymphomas.

REDUCING THE IMPACT OF PATHOGENS AND DISEASE IN THE IRISH PACIFIC OYSTER INDUSTRY TO SUPPORT THE SUSTAINABILITY AND GROWTH OF THE SECTOR (REPOSUS)

S.A. LYNCH*, B. BOOKELAAR, A. O' REILLY, S.C. CULLOTY

School of Biological, Earth and Environmental Sciences, Aquaculture and Fisheries Development Centre and Environmental Research Centre, University College Cork, Cork, Ireland

Global sustainability of the Pacific oyster *Crassostrea gigas* industry faces many challenges, in particular, mortalities associated with ostreid herpes virus-1 (OsHV-1 µVar) and *Vibrio* spp. infection. Significant losses at all stages of Pacific oyster production have occurred due to these pathogen and control measures to mitigate their impact are essential to safeguard the future growth of this commercially important oyster species. The aims of REPOSUS, a collaborative project between University College Cork (UCC), Marine Institute Ireland and National University Ireland Galway, are to ensure that the Irish Pacific oyster industry reaches its Horizon 2020 targets, by ameliorating the impact of OsHV-1 µVar and *Vibrio* spp. on this sector.

At UCC, both field and laboratory trials will be conducted to determine the role of environment and other hosts at culture sites, to help inform management and better understand how these pathogens are sustaining themselves in the environment outside their host at culture sites. The geographic extension or range of these pathogens from a culture site will also be investigated. The impact of varying environmental parameters (temperature, salinity and nutrient concentration) and combinations of those parameters on the promotion or inhibition of these pathogens in the Pacific oyster (spat, juvenile and older oysters) will be determined. The effects of plant derived antiviral compounds, which are currently used in shrimp and fish culture, will be administered to infected and uninfected Pacific oysters to determine if they inhibit OsHV-1 µVar and *Vibrio* spp. development and/or promote host immune response. On completion of REPOSUS, results achieved at UCC will provide a better understanding of the management of these pathogens and will contribute to an improvement in Pacific oyster health at all stages of production, ultimately leading to enhanced production and sustainability of the industry.

NECROSIS OF HEPATOPANCREAS IN CRABS FROM THE SEA OF OKHOTSK

T.V. RYAZANOVA*, E.A. USTIMENKO

Kamchatka Research Institute of Fisheries and Oceanography, Petropavlovsk-Kamchatsky, Russia

Several species of crabs are economically important species that are fished in the sea of Okhotsk, Russia. The most valuable of them are blue king crab *Paralithodes platypus*, red king crab *P. camtschaticus*, golden king crab *Lithodes aequispinus*, tanner crab *Chionoecetes bairdi* and snow crab *Ch. opilio*.

Diseases of these crabs were studied in the eastern area of the Sea of Okhotsk during two trapping (2006, 2010) and three trawl surveys (2007, 2012, 2013). To calculate prevalence of the diseases, we used the ratio of the number of crabs with signs of disease divided by the number of dissected animals.

We found the necrosis of hepatopancreas in all crabs species. The pathology could be found after dissection crabs only. Visual signs of disease (mummified tubule) were very similar in all cases, but microscopic examination revealed three different diseases. One of them was detect as rickettsia-like organisms infection. Rickettsia-like organisms were found in the cytoplasm of the hepatopancreas cells of the blue king crabs. The prevalence of infection in 2012 was 1.6% and in 2013 — 3.6%. Microsporidia were another cause of pathology of the necrosis of hepatopancreas. The apansporoblastic spores were detected in the cytoplasm of hepatopancreas cells. Prevalence of infection in the snow crabs in 2012 was 0.2% and in 2013 was 0.4%. Another type of disease we detect every year in all species of crabs. The prevalence of this disease is higher in the snow and tanner crabs (up to 10.8%) than in the king crabs (up to 3.6%). Gram-negative bacteria (mainly *Vibrio* spp.) were isolated from hepatopancreas and hemolymph of diseased crabs, but specific disease-causing agent remains unclear.

THE EUROPEAN PHARMACOPOEIA - ASSURING THE QUALITY OF MEDICINES

C. LANG*, C. VIELLE, S. KEITEL

Medicines need to be safe, effective and of good quality, which in an ever-changing environment represents an endless challenge.
This is why, in 1964, the Council of Europe[1] adopted the *Convention on the Elaboration of a European Pharmacopoeia*[2] to encourage member States to pool their expertise and resources in order to develop continent-wide harmonised quality standards for medicines.
The European Pharmacopoeia (Ph. Eur.)[3], which celebrated its 50th anniversary last year, lies at the heart of drug quality standards in Europe. It has the objective of progressively elaborating a single set of specifications for the quality of medicines and their components, for example active substances and excipients, providing the official standards applicable within its member countries and beyond.
It contains monographs defining the requirements for the qualitative and quantitative composition of active substances, excipients and preparations of chemical, animal, human or herbal origin, homoeopathic preparations and homoeopathic stocks, antibiotics, as well as dosage forms and containers. It also includes texts on biologicals, blood and plasma derivatives, vaccines, radiopharmaceutical preparations, and most recently, finished products. According to the Convention and EU Directives 2001/82/EC, 2001/83/EC, and 2003/63/EC[4], as amended, on medicines for human and veterinary use, its requirements are legally binding in the 37 signatory parties of the Ph. Eur. and the European Union, and are part of the regulatory requirements for obtaining a marketing authorisation for medicinal products.
Its governing body is the European Pharmacopoeia Commission, while administrative and scientific support for the Ph. Eur. is provided by the European Directorate for the Quality of Medicines and HealthCare (EDQM)[5], which is part of the Council of Europe. At the end of 2014, the Ph. Eur. covered some 2,267 monographs and 349 other texts published in its 8th Edition[6]. The Commission also counts 27 observers from all over the world, including the World Health Organisation (WHO).
In addition to its activities related to the quality of medicines, the EDQM develops guidance and standards in the areas of blood transfusion, organ transplantation and consumer health issues.

References:
1. Council of Europe (www.coe.int)
2. Convention on the Elaboration of a European Pharmacopoeia CETS No: 050 Treaty Office, Council of Europe (https://www.edqm.eu/site/1964_PhEur_Convention_Englishpdf-en-99-2.html)
3. Background and missions of the Ph. Eur. (https://www.edqm.eu/en/european-pharmacopoeia-background-50.html)
4. EUR-Lex European legislation (http://eur-lex.europa.eu/homepage.html?locale=en)
5. EDQM, Council of Europe (www. edqm.eu)
8th Edition European Pharmacopoeia (https://www.edqm.eu/en/european-pharmacopoeia-8th-edition-1563.html

ANIMAL USE IN THE QUALITY CONTROL TESTS FOR THE BATCH RELEASE OF VACCINES INTENDED FOR FISH

A. TOUT, R. COONEY*, A.M. BRADY, N. PATERSON

Immunological Veterinary Medicinal Products (IVMPs), of which vaccines form the most common class of product, benefit human and animal health by preventing and controlling infectious agents that can cause disease and death. The routine use of quality control (QC) tests during and at the end of a manufacturing process is normal for all types of medicine. However, due to their biological origin, IVMPs have inherent variability and therefore a higher potential to vary from batch to batch. Consequently, coupled with a consistent manufacturing method, production requires the routine use of strict QC tests to ensure the consistent quality, safety and efficacy of each batch before it is released onto the market. Some of the tests employed can involve the use of animals and, depending on batch size and numbers of batches released to the market, relatively high numbers of animals can be used.

The VMD regularly reviews the use of animals in the testing of veterinary medicinal products as part of its duty to ensure that animal usage is kept to a minimum and animal welfare legislation is upheld, and has recently completed a review of the numbers of animals used by manufacturers in the QC of batches of vaccines and other veterinary biological products (such as diagnostic products) released for marketing within Europe. These batches were released for use through the UK batch release authority between 2007 and 2012. This has enabled analysis of how changes in legislation have affected the number of animals used in the routine testing of veterinary vaccines and biologicals, and helped to highlight areas where efforts to advance alternative methods could be focused.

There was an 18% decrease in the numbers of animals used over the six year period examined. Some of this reduction in numbers was due to changes in test methodology where animal testing has been largely replaced by other forms of testing. It also included a reduction in the number of animals used in batch safety testing due to the removal of the requirement for safety testing for specific products; a reduction in the number of animals used to test for possible contamination of a vaccine by other extraneous agents; and a move away from more severe tests to less severe tests for potency testing was also observed.

Vaccines used for the prevention of fish diseases require a relatively large number of animals for the release of one batch of vaccine, and this is one area identified by the review where a focus is required on the development of alternative test methods.

Recent changes to legislation mean that further improvements are anticipated. In particular the removal of the legal requirement for the safety testing of every batch of veterinary vaccine released for use from April 2013 is likely to result in a significant reduction in the number of animals used for the quality control of veterinary vaccines from 2013 onwards.

EVIDENCE THAT A CITRATE-DERIVED SIDEROPHORE IS SHARED BY *PHOTOBACTERIUM DAMSELAE* SUBSP *DAMSELAE* AND SUBSP *PISCICIDA*

M. BALADO[1]*, B. PUENTES[1], J.C. FUENTES[2], J. RODRÍGUEZ[2], C. JIMÉNEZ[2], M.L. LEMOS[1]

[1]*Universidad de Santiago de Compostela, Santiago de Compostela, Spain*
[2]*Universidade de A Coruña, A Coruña, Spain*

Photobacterium damselae includes two subspecies, subsp. *piscicida* (PDP) and subsp. *damselae* (PDD) which are closely related, although they cause very different diseases. PDP is the causative agent of marine fish pasteurellosis, while PDD causes vibriosis in a variety of fish and other marine animals. A number of key virulence factors have been identified in both subspecies. Siderophore-mediated iron uptake mechanisms have proved to be among the most important virulence factors. In previous works we could identify and characterize siderophore piscibactin as the siderophore produced by European strains of PDP. However, this siderophore is not produced by non-European strains of PDP and was never detected in any PDD strain. So, the iron uptake mechanisms used by these piscibactin-negative strains remains unknown. Using proteomic and genomic approaches we have found that some PDD strains harbor a complete gene cluster for the synthesis and transport of vibrioferrin, a siderophore previously described in other vibrios. We could demonstrate that this cluster is fully functional and that vibrioferrin is in fact present in the culture media of these strains when grown in iron limiting conditions. However, this siderophore is not produced by all PDD strains and is also absent in all PDP strains examined. Thus, these PDD and PDP strains must possess another siderophore-mediated iron uptake system since they can efficiently grow in iron-limited media and siderophores are detected by both, the CAS colorimetric assay and by cross-feeding bioassays. Using a combination of proteomics and cromatographic separation techniques we are now elucidating the iron-sequestering compounds produced by these strains. Using SDS-PAGE we could detect that these strains show unique iron regulated outer membrane proteins that will be identified by peptide mass fingerprinting. In addition, we could detect an iron-regulated production of high levels of citrate and bioactive fractions obtained by liquid chromatography also show the presence of citrate containing compounds. Besides, citrate levels are much lower when other siderophore, like vibrioferrin, is being produced. Thus, a citrate-based new siderophore could be produced by PDD and we have evidences that the same siderophore could be also shared by PDP strains.

DETECTION OF *AEROMONAS SALMONICIDA* IN FISH TISSUE BY REAL-TIME PCR

S. BARTKOVA*, B. KOKOTOVIC, I. DALSGAARD

National Veterinary Institute, Technical University of Denmark, Frederiksberg C, Denmark

Disease outbreaks of furunculosis in rainbow trout freshwater farms do not occur as often in Denmark today as has otherwise been the case ever since the 1950s when the causative agent of furunculosis, *Aeromonas salmonicida* subsp. *salmonicida*, was first discovered in Denmark. However, even though the trout are vaccinated before transfer out to the sea, outbreaks of furunculosis continue to occur at sea during elevated temperatures. It has thus been speculated that fish could be carrying the bacterium as a latent infection from the freshwater to the sea.
Several PCR and qPCR assays for detection of *A. salmonicida* have been developed in the past. Nevertheless, most of these assays either lack high sensitivity in tissue or have only been tested on pure culture and/or few tissue types. Moreover, in cases where carrier fish were investigated, laborious and slow enrichment steps were needed in order to acquire a positive result. A sensitive, specific, rapid and cost-effective assay for detecting *A. salmonicida* in carrier fish is thus still needed.
In this study, a highly sensitive and specific real-time PCR has been developed, based on previous research by Balcazar et al. (2007). The assay uses a self-quenched fluorogenic primer set designed from a DNA probe sequence for *A. salmonicida*, which is the most frequently used target for species-specific *A. salmonicida* molecular methods to date. Thus far the assay has been tested on five different rainbow trout tissue samples (gills, kidney, brain, intestine, spleen) spiked with various *A. salmonicida* dilutions, without showing signs of inhibition. Preliminary results show that the assay has a higher sensitivity compared to traditional bacterial methods and both methods are currently being compared in fish from natural occurring outbreaks and fish without clinical signs of infection. Key results from both methods will be presented and discussed.

HAEMOAGGLUTINATION, HAEMOLYTIC AND CYTOTOXIC ACTIVITY OF *AEROMONAS VERONII* BIOVAR SOBRIA

T. BOSSÙ*, S. MAROZZI, S. AMITI, G. DANTE, A. ALTIGERI, G. CARDETI

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, M. Aleandri, 1411, Via Appia Nuova 1411 – 00178 Rome, Italy

Five strains of *Aeromonas veronii* biovar sobria isolated from clinical cases of fish disease, were examined for cytotoxic, haemoagglutination and haemolytic activity. The haemolytic activity was performed on cultured *Aeromonas veronii* strains onto solid blood agar medium. Cytotoxicity of the isolates was tested on confluent monolayers of the fish cell line EPC (epithelioma papulosum cyprini) grown in 24-well tissue culture plates in cell culture medium EMEM supplemented with 10% fetal bovine serum, 1% (wt/vol) Lglutamine, 1% (wt/vol) antibiotic-antimycotic solution. *Aeromonas veronii* biovar sobria strains were incubated in Brain Heart Infusion Broth (BHI) at 37°C for 24 h and then centrifugated at 3000 rpm for 10 minutes at 22°C and the serial dilutions of the supernatants were performed. The EPC monolayers were than exposed to 100 µl of these dilutions for 6 h at 22°C. The cytotoxic activity was measured as rounding up, detachment, and loss of viability of the cells, as seen under a light microscope within 6 hours.

Haemagglutination (HA) tests were performed both on glass slides and on microtiter plates. The microtiter plates test was performed at room temperature, placing 20 µl of *Aeromonas veronii* biovar sobria culture in BHI washed twice in PBS (Phosphate Buffer Saline), into the well of the first row. Subsequently 20 µl of PBS were added in all the wells of the microplate. Doubled serial dilutions (1: 2) were done. Finally 40 µl of a 3% (vol/vol) suspension of erythrocytes from sheep, rabbit, chicken, fish (*Oncorhynchus mykiss*) in PBS, were placed in each well. A stereomicroscope was used to determine the HA titers.

The HA test on glass slides were performed by mixing a loopful of bacteria with a 3% (vol/vol) suspension of erythrocytes from sheep, rabbit, chicken, fish (*Oncorhynchus mykiss*) in PBS, on glass slides. Strains were considered HA-negative if agglutination did not occur within 5 min.

All the strains were able to haemoagglutinate fish blood (*Oncorhynchus mykiss*) but only one isolate caused HA of sheep (titer 1:8), rabbit (titer 1:4) and chicken (titer 1:2) blood. The haemolytic activity performed onto blood agar was a true lysis of red blood cells (β haemolysis). Four of the five strains had cytotoxic activity on EPC cells.

Our results suggest that *A. veronii* biovar sobria may be an underestimated causative agent in fish pathology.

EVALUATION OF INNATE DEFENCE STATUS ON *VIBRIO* SP. INFECTED SEA BASS (*DICENTRARCHUS LABRAX*) UNDER INTENSIVE CULTURE IN ALGERIA

R. CEREZUELA[1], D. MOKRANI[2], M. OUMOUNA[3], M.A. ESTEBAN[1], A. CUESTA[1]*

[1]*University of Murcia. Murcia, Spain*
[2]*Université de Blida, Blida, Algeria*
[3]*Université de Médéa, Médéa, Algeria*

Algeria is trying for many years to diversify its aquaculture production in quantity and quality, the current trend is probably the development of marine fish farming structure, such as European sea bass (*Dicentrarchus labrax*). The aim of the present work was to study the effects of bacterial contamination on the immune response in sea bass specimens from the ONDPA farm (Office National Aquaculture Development and Protection). The ONDPA farm, located 77 km from Algiers and 30 km east of the province of Boumerdes, in the town of Djinet, is an Algerian-Spanish joint venture that intensively produces gilthead seabream (*Sparus aurata*) and sea bass in ponds fed by heated sea water as a result of rejection of a nearby power plant. Fish culture in hot water offers real advantages (in terms of fish growth) compared to farms in natural seawater temperature, but the increase in temperature favors the development of certain pathogens including vibriosis, forcing to optimize disease prevention methods. For this work, twenty fishes from the ONDPA farm, 5 apparently non-infected and 15 with typical lesions of vibriosis were sampled. Bacteriological analysis isolated and identified *Vibrio alginolyticus* and *V. hollisae* in the diseased fish specimens. Innate immune status was investigated in serum samples by total IgM levels, peroxidase, protease, antiprotease and bactericidal activities against *V. anguillarum* and *Escherichia coli*, as well as the blood expression of selected immune-related genes. Our results showed no statistical differences between the control and infected fishes on IgM levels, peroxidase, protease or antiprotease activity. However, the bactericidal activity against *V. anguillarum* was significantly lower in infected fishes compared to the control group.

Financial support by grants AGL2013-43588-P (MINECO and FEDER) and 04538/GERM/06 (Fundación Séneca, Spain) is gratefully acknowledged.

DEVELOPMENT OF CHALLENGE MODEL TO PASTEURELLOSIS IN MEAGRE (*ARGYROSOMUS REGIUS*)

T. Baptista[1]*, F. Galhano[1], A.M. Pombo[1], J.Z. Costa[2], M.M. Sampaio[1]

[1]*MARE-GIRM, IPLeiria, (Portugal)*
[2]*Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, UK*

Meagre *Argyrosomus regius* is a new species for the Mediterranean aquaculture. *Photobacterium damselae* subp. *piscicida (*Phdp) is a well known pathogen and can induce high mortalities in meagre (J. Z. Costa, *personal comm*.). Since Phdp has a high prevalence in the Mediterranean region it is important to understand the susceptibility of meagre to this specific pathogen. The aim of this study was to test the susceptibility of meagre to distinct doses of *Photobacterium damselae* subp*. piscicida*. In order to determine the LD_{50}, meagre (weighing 28.3±1.6g) from a commercial farm, were intraperitoneally injected (i.p.). The volume injected was 100µl and were administered three doses of $1.1x10^4$, $1.1x10^5$, $1.1x10^6$ CFU ml^{-1} of *Photobacterium damselae* subps. *piscicida* cultured on TSA (+1,5% salt) and suspended in Hank´s solution. Control fish were i.p. with Hank´s solution. The infectious dose used was based on optical densities of Phdp suspensions and confirmed by serial plating for determination of CFU. The study was performed with four systems with flow trough seawater, triplicate tanks for each pathogen's dose and for control group, and fish density in each tank was 6.8 gm^{-3}. Water temperatures were maintained between 22-24ºC, salinity 35 ppm and photoperiod of 12 light : 12 dark. Mortality was monitored daily during three weeks post challenge and moribund fish were analyzed and samples collected for re-isolation of the pathogen and identification by specific PCR (Osorio *et al*., 2000). The results show that meagre is susceptible to the Phdp with bacteria recovered from spleen, kidney and liver, by microbiological methods and PCR. The mortality allowed determining a LD_{50} value for the infection of $2.3x10^5$ CFU ml^{-1}. Mortality occurred within the first 4 days post infection. Further studies are ongoing with the development of a cohabitation infection model.

PENGUIN: AN IMAGE ANALYSIS PROGRAM TO MEASURE CHANGES IN COLONY SIZE OF FISH ASSOCIATED BACTERIA

S.J. HARRIS[1, 2*!], T. SPECHT[3!], S.L. TAYLOR[1], D. HOOLE[1]

[1]*Keele University, Keele, UK*
[2]*University of Veterinary Medicine, Hanover, Germany*
[3]*The Woodpecker Project, Birmingham, UK*
[!]*Equal contribution*

The cost of specialist equipment to accurately measure differences in bacteria colony sizes can be prohibitively expensive, whereas cheaper options, such as measuring by eye, may sacrifice accuracy. To resolve this, a collaboration was established between Keele University and The Woodpecker Project to create a cost effective means of accurately measuring the size of bacteria colonies from fish and water.

The program "PlatE aNalysis proGram UsINg pixels to measure bacteria colony size" (PENGUIN) is a web based program written using PHP and is hosted on a cloud based server. It registers over 16 million individual colours from the RGB colour palette (255^3), and can accurately detect a single pixel that is a "new" colour when compared to a base image. PENGUIN quantifies bacteria growth as the number of pixels that are a new colour in comparison to images of the same plate before any colonies have grown.

The data presented shows optimisation of a standard protocol for taking images of plates before and after bacteria growth using *E. coli* and a standard point and click camera set up. After testing various parameters including different light sources, exposure times and rotation of plates to avoid false positives caused by shadows, power analysis shows comparing 8 pre-growth and 8 post-growth images per plate will accurately determine the size of bacterial colonies as measured in pixels.

PENGUIN is owned by The Woodpecker Project and developed in association with Keele University.

IN VITRO ANALYSIS OF THE EFFECT OF β-GLUCANS ON GROWTH AND SURVIVAL OF FISH ASSOCIATED BACTERIA

S.J. HARRIS[1, 2]*, A. HUGHES[1], J. NATALELLO[1], T. SPECHT[3], D. HULSE[1], D. HOOLE[1]

[1]*Keele University, Keele, UK*
[2]*University of Veterinary Medicine, Hanover, Germany*
[3]*The Woodpecker Project, UK*

Bacteria play a significant role in the life of a fish whether as a symbiont, a co-habitor of water or as a pathogen. It is therefore important to consider the impact of any products used by farmers/keepers to maintain fish health upon the prevalence of associated bacteria species. MacroGard® is a commercially available β-1,3/1,6-glucan product commonly used in feeds as an immunomodulant for a wide variety of fish including farmed and ornamental species. Predominantly, oral administration is used and may affect the symbiotic microbial population within the gut and potentially the environmental microbiome through uneaten feed or undigested remains in faecal matter. The effect of MacroGard® on the growth and survival of individual bacteria species *in vitro* was investigated. Using individual colony counts, minimum inhibition concentration (MIC) assays, fatty acid production under anaerobic fermentation conditions, and comparison of colony sizes, several bacteria species were exposed to varying concentrations of MacroGard® and their growth/survival measured. The reference strain *Aeromonas salmonicida* ssp. *salmonicida* (NCIMB 1102), a potential pathogen of the common carp (*Cyprinus carpio*), was shown to be unaffected by the presence of MacroGard® indicating there would be no risk of increasing infection caused by an higher *A. salmonicida* subsp. *salmonicida* numbers when MacroGard® is included in the feed. The same effect was seen with the non pathogenic reference strain *Bacillus subtilis* subsp. *spizizenii* (NCIMB 8054). In contrast to both reference strains, isolates taken from water samples and the intestine of carp have been shown to either have inhibited growth or to be able to flourish in the presence of MacroGard®. Whilst it is important to understand the impact of products such as MacroGard® on the general microbial population, the effect on individual bacterial species, some of which may be pathogenic, is also important.

USING *IN SILICO* SEQUENCE DATA TO ESTIMATE THE EFFECTIVENESS OF GENUS SPECIFIC PRIMERS ENCODING FOR THE 16S rDNA GENE IN BACTERIA TYPICALLY FOUND IN FISH

S.J. Harris[1, 2]*, D. Hoole[1]

[1]*Keele University, Keele, UK*
[2]*University of Veterinary Medicine, Hanover, Germany*

Quantitative PCR assays are increasingly common ways of assessing changes in the presence of a particular bacteria genus or species within a sample. The gene of choice for many of these assays is the 16S rDNA gene, which is a highly conserved housekeeping gene present in all species. This commonality means individual primer pairs can be used to measure across a very broad spectrum of diversity however the gene is not so highly conserved that it would prevent identification to species or strain level.
One of the challenges faced when designing primers for a specific genus is finding regions that are conserved only in the target species. A model has been developed to estimate the likelihood of a primer binding to a sequence based upon number of errors and position. Up to 3 errors within the sequence are accepted for analysis with errors closer to the 3' end of the primer being considered as more likely to impact binding affinity.
To test this model, previously published primers for different genera including *Aeromonas*, *Lactobacillus and Pseudomonas* and the family *Enterobacteriaceae* were compared against an *in silico* library of 16S sequences for bacteria commonly found in the gut of the fish species *Cyprinus carpio* to estimate the likelihood of cross reactivity. This will then be confirmed *in situ* utilising DNA isolated from multiple bacteria species corresponding to the sequences analysed.

FIRST ISOLATION OF *TENACIBACULUM DICENTRARCHI* FROM DISEASED ATLANTIC SALMON (SALMO SALAR) CULTURED IN CHILE

P. ILARDI[1], C. SANDOVAL[1], R. AVENDAÑO-HERRERA[2, 3, 4]*

[1]*Veterquímica S.A., Research and Development Laboratory, Santiago, Chile*
[2]*Universidad Andrés Bello, Viña del Mar, Chile*
[3]*Interdisciplinary Center for Aquaculture Research (INCAR), Chile*
[4]*Centro de Investigación Marina Quintay (CIMARQ)*

Tenacibaculum maritimum, *T. ovolyticum*, *T. discolor*, *T. gallaicum*, *T. soleae* and *T. dicentrachi* have been described as pathogens for several fish species, some of which are serious concern for marine aquaculture. Chile is currently the second largest producer of farmed salmon in the world. In Summer 2010, Atlantic salmon (*Salmo salar*) farmed in marine cages near to Puerto Montt (Chile) showed severe destruction of tail rots and frayed fins, and sometimes damage on the gills. Bacteriological analysis of these lesions revealed the presence of a Gram-negative and filamentous bacterium. A polyphasic taxonomic analysis was performed to identify *T. dicentrachi* and its pathogenic potential for farmed fish were investigated in experimental challenges. Based on this characterization, we report the first isolation of *Tenacibaculum dicentrachi*, from a farmed population of Atlantic salmon in Chile.

FONDAP/CONICYT 1511002.7

GENOMICS AND PROTEOMICS OF *PISCIRICKETTSIA SALMONIS*: PATHOGEN-HOST INTERACTIONS AND ANTIBIOTICS RESISTANCE

J. FIGUEROA[1, 2]*, D. HAUSSMANN[1, 2], F. LAGOS[2], A ISLA[2], L. SOTO[1], M. MUÑOZ[1, 2], A. ROMERO[1, 2], A. YAÑEZ[1, 2]

[1]*Universidad Austral de Chile, Valdivia, Chile*
[2]*Centro FONDAP, Interdisciplinary Center for Aquaculture Research INCAR, Concepción, Chile*

P. salmonis is an important pathogen of the Chilean salmon industry, therefore massively sequenced nine genomes including type strain LF-89. Automatic annotation was performed with the GenDB *on line* server, which allowed us to identify ~3600 genes and a function was assigned to each gene. The genome has a percentage of G/C ~39% and 50% of the genes identified correspond to the subsystems associated with cell wall, cofactors, prosthetic groups, pigments, vitamins, metabolism of proteins, carbohydrates, RNA and miscellaneous genes.
Global expression patterns were analyzed using proteomic and transcriptomic to identify genes and proteins involved in pathogenicity and virulence. 2D-DIGE proteome was used for the two culture conditions and the proteome and transcriptome of a highly virulent field strain (IBM-40) and the type strain LF-89. The comparative analysis between strains showed differential expression of various genes, highlighting components of type IV secretion system and virulence factors associated with *purl, acrab* and *ahpc*. The comparative proteomic analysis of LF-89 strain, in terms of intracellular life and cell-free medium, showed differential expression of virulence as global regulators and PNPase CsrA.
We have developed new culture media for this bacterium useful for determining antimicrobial susceptibility by minimal inhibitory concentration (MIC) for Florfenicol and Oxytetracycline and the field strains analyzed have different sensitivity to both antibiotics. Therefore constitutes an important contribution to help elucidate the effect on gene expression differences in intracellular survival, pathogenicity and virulence of *P. salmonis.*

FONDECYT-1130069, FONDAP (INCAR)-15110027.

FIRST RECORD OF *STAPHYLOCOCCUS HOMINIS* FROM THE CULTURED GILTHEAD SEA BREAM (*SPARUS AURATA*) IN TURKEY

J. KORUN[1,]*, M. YıLMAZ[1], M. GÖKOĞLU[1], S.Y. BULGUROĞLU[1]

[1]*Akdeniz University Faculty of Fisheries, Campus, Antalya-Turkey*

Mediterranean aquaculture is increasingly growing and gilthead sea bream (*Sparus aurata*, L.) is the most cultured fish species in countries such as Greece, Italy, Spain, and Turkey which has a coastline along the Mediterranean Sea. Because of it's economical important and demand for human consumption, many researchers on both wild and cultured gilthead sea bream were carried out. These include feeding, genetic, biology, and diseases caused by infectious agents and noninfectious disorders. The most important bacterial infections affecting gilthead sea bream culture are photobacteriosis (previously known as pasteurellosis), flexibacteriosis, pseudomonadiasis ('winter ulcer'), and vibriosis. Causative agents of these infections are Gram-negative bacteria. Among the Gram-positive bacterial species, some *Streptococcus* species such as *Streptococcus iniae* and *S. agalactiae* and *Staphylococcus* species including *Staph. cobuii*, *Staph*. *lentus*, *Staph*. *schleifer* and *Staph*. *warneri* from sea bream were isolated and reported by different authors. A disease outbreak was observed on the cultured sea bream from a commercial marine fish farm which was located in the Gulf of Antalya, the Mediterranean Sea shore of Turkey in June 2014. Moribund ten fish with clinical sings were randomly collected from the net cage. The fish sizes ranged from 150 to 240 g and sea water temperature was 26 °C. Inoculations were prepared from internal organs such as liver, spleen, and kidney, and inoculated onto Brain Hearth Infusion agar (BHI) supplemented with 1.5% NaCl. The inoculated Petri dishes were incubated at 26 ± 2 °C for 72 h. After incubation, bacterial colonies were subcultured and the isolates were identified using by PCR. According to the PCR assay results, *Staph. hominis* strains were firstly identified. *Staph*. *hominis* from unpolluted areas of the Sea of Marmara in Turkey was reported; however, *Staph*. *hominis* was not isolated from the diseased cultured and/or wild gilthead sea bream and also another fish species in our country until this time.

ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES ACTIVE AGAINST *YERSINIA RUCKERI*

S. MAROZZI*, F. DI GIAMBERARDINO, G. MIGLIORE, S. COLONNA, S. SITTINIERI, G. CARDETI, T. BOSSÙ

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, M. Aleandri, Via Appia Nuova 1411 – 00178 Rome, Italy

Bacteriophages are viruses of bacteria and archaea. The infection by a lytic phage results in the lysis of the bacterial cell and release of a new phage progeny.
This work was carried out in order to isolate bacteriophages active against *Yersinia ruckeri*, the causative agent of enteric redmouth disease (ERM). Five samples of water were taken from three raceways in wich trout (*Salmo trutta*) and carp (*Cyprinus carpio*) were being cultured. Samples were filtered with 0.22µ filter and then inoculated in double-strength Luria Bertani Broth containing 2mM $CaCl_2$ with an overnight culture of *Yersinia ruckeri* strain isolated from fish (*Cyprinus carpio*). Subsequently, the supernatant of each samples was placed into two test tubes. One of this was additionated with chloroform (1:100) and stored at 4° C while the second was used to test the phage activity against *Yersinia ruckeri* with two spot test methods using Luria Bertani agar, Luria Bertani soft agar and Luria Bertani Broth cointaning 10mM $CaCl_2$.Two samples were positive and therefore the supernatants with chloroform (1:100) were tested to determinate the infectious phage particles using the Double Agar Overlay Plaque Assay. Plates were incubated at 30°C for 24 h. Subsequently 4 phages were purified by removing a well isolated plaque and plating it using the Double Agar Overlay Plaque Assay. In order to ensure a single phage-strain population the purification procedure were repeted for three times. The pahges were then characterized using negative staining transmission electron microscopy (EM). Finally their effectivness against 5 other strains of *Yersinia ruckeri* and 5 strains of *Yersinia enterocolitica* was tested.
Bacteriophages active against *Yersinia ruckeri* were detected in two of the five tested samples (40%). The numbers of the infectious phage particles were respectively $2{,}4x10^8$ UFP / ml and $2{,}0x10^7$ UFP / ml. The 4 purified plaque showed the same morphology and were characterized by EM as tailed phages belonging to the order *Caudovirales,* family *Podoviridae*.
At least all the phages were effective against 2 strains of *Yersinia enterocolitica* and 2 strains of Y*ersinia ruckeri*. Other tests will be performed to evaluate the host range and stability of phages.

PERSISTENT *STREPTOCOCCUS AGALACTIAE* CELLS INDUCED BY FLORFENICOL *IN VITRO* AND *IN VIVO* IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

T.F. OLIVEIRA[1], G.A. QUEIROZ[1], H.C.P. FIGUEIREDO[1, 2], C.A.G. LEAL[1, 2]*

[1]*AQUAVET, Laboratory of Aquatic Animal Diseases, Federal University of Minas Gerais, Belo Horizonte, Brazil*
[2]*AQUACEN, National Reference Laboratory for Aquatic Animal Diseases, Ministry of Fisheries and Aquaculture, Federal University of Minas Gerais, Belo Horizonte, Brazil*

S. agalactiae is one of the most important pathogens for tilapia farming worldwide. The main control measure applied in cases of streptococosis is the antibiotic therapy, being the florfenicol (FLO) a drug commonly used in several countries. In Brazil, therapeutic failures have been reported with the use of this antibiotic in tilapia farms. Persistence induced by antimicrobials has been described for pathogenic streptococci of mammals. This phenomenon has never been described for fish pathogens. The aims of this work were to evaluate the efficacy of florfenicol to control *S. agalactiae* infection in Nile tilapia fingerlings and the occurrence of *S. agalactiae* persistence. *S. agalactiae* strain SA95 was used in efficacy trials and *in vitro* assays. The MIC of FLO for SA95 was determined according to CLSI, resulting in a MIC value of 1µg/ml. Nile tilapia fingerlings were i.p. challenged and orally treated with FLO at the doses of 10, 20, 30 and 40 mg per kg of body weight, for 10 consecutive days. The FLO at doses of 30 mg e 40 mg was efficient to control the disease, with 90% and 100% of survival, respectively. However, 30% and 10% of treated fingerlings with 30 and 40 mg of FLO were positive in the bacteriology of brain. To address if that results were caused by persistent *S. agalactiae* cells, persistence induced by FLO was evaluated according to the method described by Willenborg *et al.* (2014) with some modifications. The strain was inoculated into BHI broth until reaches the stationary phase. The bacteria was inoculated in Mueller Hinton broth containing 100 µg/ml of FLO (100x the MIC value) at final concentration of 10^7 CFU/ml and incubated at 28°C. Bacterial counting were performed 1, 2, 4, 8 and 12 hours post-inoculation. After 1hour, bacterial concentration decreased to 10^4 CFU/ml and maintained that concentration until 12 hours of incubation. SA95 showed a persistence phenotype. FLO was able to control the mortalities caused by *S. agalactiae* in Nile tilapia, however, persistent cells induced carrier state in treated fish. This is the first description of persistence induced by antibiotic in fish pathogenic *S. agalactiae*.

PERICARDITIS IN SALMON BROOD FISH IN WESTERN NORWAY

S. GISKEGJERDE*, R.H. LYNGSTAD, S.M.R. NYGAARD

FoMAS – Fiskehelse og Miljoe as, 5680 Lundegrend, Norway

Salmon Brood fish aquaculture is one of the heavily controlled practices in the Norwegian Aquaculture industry. According to aquaculture management regulations, every single fish that succumbs starting from a minimum of 9 months before stripping is autopsied by an aqua health biologist or a veterinarian. In addition, autopsy is performed on every spawning fish during stripping. Brood fish sampling is carried out based on demands from the government, from the farming company and according to customer specifications. Risk based sampling and screening is also performed in accordance to legislations.

During the 2013 and 2014 stripping seasons, post-mortem examinations revealed pericarditis in a considerable number of recently dead and seemingly healthy brood fish. Bacteriological samples were taken from diseased fish and sent to the Norwegian Veterinary Institute in Bergen for culturing. Bacterial cultures showed massive growth of bacteria with the morphological characteristics consistent to *Carnobacterium* spp. *Carnobacterium* spp. is known for causing infections on serous membranes, particularly in brood fish.

FoMAS – Fiskehelse og Miljoe AS is a fish health company located in the Western part of Norway. It consists of veterinarians and fish health biologists who perform health control on several fish farms, among other two brood stock producers.

Prevalence of pericarditis between groups of Brood fish and also risk factors will be further discussed.

VIRULENCE GRADING OF *VIBRIO* SPP. STRAINS BY EXPERIMENTAL CHALLENGE OF ATLANTIC HALIBUT (*HIPPIGLOSSUS HIPPOGLOSSUS* L.), ATLANTIC COD (*GADUS MORHUA* L.) AND TURBOT (*SCOPHTHALMUS MAXIMUS* L.) YOLK SACK LARVAE

A. RØNNESETH[1]*, P. D'ALVISE[2], Ø. TØNNESEN[1], G.T. HAUGLAND[1], T. GROTKJÆR[2], K. ENGEL-SØRENSEN[3], L. NØRREMARK[3], L. GRAM[2], Ø. BERGH[4], H.I. WERGELAND[1]

[1]*University of Bergen, Bergen, Norway*
[2]*Technical University of Denmark, Lyngby, Denmark*
[3]*Fishlab, Hojbjerg, Denmark*
[4]*Institute for Marine Research, Bergen, Norway*

A high and stable production of marine larvae has long been the bottleneck in intensive farming of marine fish species. One of the major challenges is bacterial diseases. High organic load and live feed organisms added in high concentrations in rearing systems contribute to possible introduction and excellent growth conditions for many bacteria. We have experimentally challenged yolk sack larva of cod, halibut and turbot with 38 different isolates of *Vibrio* spp., most isolates were of the species *Vibrio anguillarum*. The strains comprised different serotypes and were isolated from different fish species. The aim of the experiment was to test the virulence of the strains on larvae from different fish species. The results surprisingly showed that a few strains clearly stood out as highly virulent to all fish species. This work will further be used to test strategies for probiotic treatment of fish larvae as a mean to reduce the use of antibiotic treatment in these life stages.

PHYLOGENETIC AND VIRULENCE ANALYSIS OF *AEROMONAS VERONII* ISOLATED FROM FRESHWATER FISHES IN HUNGARY

B. SELLYEI[1]*, Z.S. VARGA[1], P. PAULUS[2], M. PAPP[2], K. MOLNÁR, [1] CS. SZÉKELY[1]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*National Food Chain Safety Office, Veterinary Diagnostic Directorate, Budapest, Hungary*

Bacterial infections, caused by motile members of the genus Aeromonas, are among the most common and troublesome diseases of fish raised in ponds and recirculating systems. These bacteria are widespread in the aquatic environment, since they are capable of utilizing nutrients present in water and surviving for long periods in the absence of the host, as well. Whether acting alone or in mixed infections with other organisms, they are responsible for the great variety of infections from the skin ulcer, superficial or invading deeply into the muscle, to the internal systemic disease (septicaemia). All species of fish, scaled and unscaled, are susceptible to infection.
Most frequently the *A. hydrophila*, *A. caviae* and *A. veronii* species induce disease in fishes. With great host and virulence ranges and with considerable zoonotic ability the A. veronii possesses. Moreover the increasing water temperature resulting from climate change enhances effectively its prevalence and infection intensity. For the delineation of presumed clonal subtypes with different pathogenicity is needed the exhaustive knowledge of the population.
Thus, the aim of our study was to detailed molecular analysis of *A. veronii* strains isolated from ulcerous skin, affected fins, and internal organs with lesions of wild and cultured freshwater fishes in Hungary. Following the genus-specific identification and species-specific classification carried out with PCRs, sequences of some housekeeping gene (16S ribosomal RNA, *cnp*60 - type I chaperonin, *gyr*A, *gyr*B - α, and β-subunit of DNA gyrase, *rpo*B - β-subunit of DNA-dependent RNA polymerase, and *dna*J - heat shock protein 40) were analysed for revealing their phylogenetic relations. In addition, the occurrences of the most prevalent virulence factors: lateral flagella, DNase, nuclease, serine protease, lipases involved the tissue invasion and toxins (entero-, haemolytic toxins) were detected. Comparison of generated different results indicated the existence of potential subgroups. Estimation of their virulence abilities required further in vitro pathogenicity assays.

This project was supported by the: KTIA-AIK-12-1-2013-0017 project, the Hungarian Scientific Research Fund (OTKA K 100132, OTKA PD 101091) and the 'János Bolyai' Research Scholarship of the HAS to B. Sellyei.

COMPARISON OF PROTEOME TYPE AND SEROTYPING FOR *STREPTOCOCCUS PARAUBERIUS* FROM OLIVE FLOUNDER (*PARALICHTHYS OLIVACEOUS*)

S.W. Kim, J.S. Lee, S.P. Im, J.M.S. Lazarte, T.S. Jung*

Laboratory of Aquatic Animal Diseases, College of Veterinary Medicine, Gyeongsang National University, Jinju, 660-701, Korea

The olive flounder, *Paralichthys olivaceus*, is highly important marine culture species in Korea and Japan. However, several bacterial pathogens caused severe mortalities in farmed fishes, especially *Streptococcus parauberis*. In this study, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI TOF MS) was applied to evaluate the characteristics of proteome then serological differentiation was also conducted in parallel. 145 of *S. parauberis* isolates were collected from diseased olive flounders from 2003 to 2008 from Jeju Island in South Korea. Serological study was able to discriminate between serotype I and II at the ratio of 62% and 37%, respectively. In the proteome type analysis, the isolates were divided by two clusters, cluster 1 occupied 43% and cluster 2 showed 57%. Cluster 1 showed to be identical with serotype I (100%), whereas cluster 2 was included two serotypes, serotype I (33%) and II (65%) respectively. Furthermore, specific peaks were able to discriminate between serotype I and II within cluster 2. In this study, MALDI TOF MS analysis was proved as an alternative method for rapid and reliable identification of fish pathogens *S. parauberis*.

Ref: Marine Biotechnology, 2013, Vol. 15, Issue 3, pp 340-348

BACTERIOLOGICAL ASSESSMENT OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) REARED IN TWO DIFFERENT TECHNOLOGIES

E. TERECH-MAJEWSKA[1], J. PAJDAK[1], B. KAZUŃ[2], K. KAZUŃ[2], E.KACZOREK[1], E. SZCZUCIŃSKA[1], M. ZEMBRZUSKA[1], A.K. SIWICKI[2]*, J. SZAREK[1], K. SKIBNIEWSKA[1]

[1]*University of Warmia and Mazury, Olsztyn, Poland*
[2]*Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland*

Bacteriological tests of fish in farming cycle are the basis of fish health evaluation. They are performed with health crisis and when increased fall is noted. These tests are part of comprehensive quality evaluation of fish in two different systems: open water flow system (OS) and closed water system (RAS, recirculatory aquaculture system). Knowleadge of bacteriological profile and monitoring inhibition of potential pathogens during intensive farming as well as environment changes may have great influence for danger evaluation and prophylaxis.
Evaluation was conducted on healthy fish (lack of any significant differences from the physiological condition) in groups according to body mass: S from 300 g to 500 g and B from 501 g to 800 g, during spring and autumn (2010 – 2012). The assessment was performed on total 960 fish originated from 6 fish farms (3-OS and 3-RAS). Every time 40 fish were taken for examination. The samples (5 from each fish) were collected for bacterio Amies Medium (and kept in 4^0C). The bacteriological tests were performed using routine biological methods. Cultured bacteria were identified with the API test (BioMerieux, Poland).
There were no differences in the pathogenic flora of investigated fish from the same farm but they were present in different body mass groups (S and B). On all the farms with either OS or RAS rearing systems, *Aeromonas* (*A. hydrophila*) and *Pseudomonas* (*P. fluorescens*) were isolated. Obligate fish pathogen - *Aeromonas salmonicida* was detected in internal organs of trout, mostly during spring on 2-OS and 3-OS farms and gills on 3-OS, 2-RAS farms.
The results confirm that while testing diagnostically the attention should be paid to the presence of potentially pathogenic bacteria in the internal organs, especially when anatomopathological (macroscopic) changes are noted. On all OS and RAS farms saprophytic flora was isolated during spring and autumn. The above-mentioned bacteria show proteolytic nature and their presence may influence not only fish health but also technologic quality of rearing.

The research has received funding from the EU and Ministry of Agriculture and Rural Development (Poland) - under grant "PO Fish 2007 -2013, agreement no. 00001-61724-OR1400002/10".

FIRST REPORT OF MIXED MYCOBACTERIAL INFECTION IN SILVER AROWANA (*OSTEOGLOSSUM BICIRRHOSUM*)

K. VARELLO*, M. RIGHETTI, V. AUDINO, M. PEZZOLATO, E.A.V. BURIOLI, C. FOGLINI, M. BOTTA, E. BOZZETTA, M. PREARO

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

The silver arowana (*Osteoglossum bicirrhosum*) is a tropical freshwater fish of the family *Osteoglossidae* native to South America. It is commonly kept in aquaria but in the Amazon region has also economic significance as a food fish. No reports of mycobacterial infections in this species are found in bibliography.
The aim of the present work is to describe the first case of mixed mycobacterial infection in a silver arowana.
In December 2014 an adult male silver arowana specimen was sent for post-mortem investigations at the Fish Diseases Laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin.
At necropsy, no visible lesions in the visceral organs were observed. On the basis of the standard protocol for ornamental fish, parasitological, histopathological and bacteriological investigations, including mycobacterial culture, were performed.
Liver, spleen and kidney were collected and partly fixed in 10% buffered formalin for the histological examination and partly, not fixed, utilized for the other analyses.
Parasitological investigations resulted negative. *Aeromonas hydrophila* was isolated and identified from kidney.
From all samples, with the exception of kidney, it was possible to isolate non-chromogenic colonies and the purified isolates were characterised by phenotypical and biochemical identification. The isolates were identified as *M. abscessus* and *M. fortuitum* both in spleen and in liver. Molecular investigations are currently underway.
At histopathological examination, granulomas were detected on liver and spleen. No microscopic lesions were found in kidney. Granulomas presented a central necrotic area surrounded by inflammatory cells and enclosed by a thin capsule. All granulomas were ZN positive with mild to moderate numbers of acid-fast bacilli in the necrotic centres and in macrophages.
To our knowledge this is the first report of mycobacterial infection in this fish, moreover caused by 2 different species belonging to different complex.

CHARACTERISATION AND ANTIBIOTIC RESISTANCE OF HUNGARIAN FRESHWATER *FLAVOBACTERIUM* ISOLATES

ZS. VARGA[1], B. SELLYEI[1]*, P. PAULUS[2], M. PAPP[2], K. MOLNÁR[1], CS. SZÉKELY[1]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*National Food Chain Safety Office, Veterinary Diagnostic Directorate, Budapest, Hungary*

The aim of this work was to study the changes induced by increasing water temperature resulting from climate change in the prevalence of *Flavobacterium columnare* in wild and cultured freshwater fishes.
Using selective cytophaga agar and a species-specific PCR designed by Bader et al. (2003), we identified as *F. columnare* 25 isolates collected from ulcerous skin, eyes, gills and inner organs with lesions and from the skin or gills of healthy fishes. The genotypes of isolates were determined using PCR-RFLP according to Darwish et al. (2005); however, the electrophoretic profiles obtained were different from those published earlier. Twenty isolates were identical with each other, further four strains varied from the former only in restriction fragment profile with one or the other enzyme, while one isolate produced a different fragment profile with both enzymes.
Sequencing of 1360 bp long fragments of the 16S rRNA gene confirmed the discrepancy. Twenty-three isolates showed 97–99% identity with *F. johnsoniae*, a species closely related to *F. columnare*. The remaining two isolates were identified as *Chryseobacterium piscium* and an unnamed *Chryseobacterium* genomospecies.
The antibiotic resistance patterns of the isolates were determined by Kirby-Bauer disc diffusion susceptibility test against 10 antibiotics. All isolates were multiresistant, i.e. they showed resistance against at least four antibiotics. The majority of resistant isolates presented complete resistance to some antimicrobial agents, which made the estimation simple. All 25 isolates showed resistance against ampicillin and polymyxin B, the 23 *F. johnsoniae* strains proved to be resistant to cotrimoxazole, while only one *F. johnsoniae* and one *Chryseobacterium* sp. strain each was sensitive to gentamicin. High rates of resistance were present within the isolates against chloramphenicol (19) and oxytetracycline (17), as well. In contrast with these results, erythromycin (22), enrofloxacin (21), furazolidone (19) and florfenicol (17) were highly effective against flavobacteria.
The high-level multiresistance demonstrated in this study hampers medical treatment and can play a major role in the transmission of resistance genes from environmental to pathogenic bacteria.

This project was supported by the KTIA-AIK-12-1-2013-0017 project, the Hungarian Scientific Research Fund (OTKA K 100132, OTKA PD 101091) and the 'János Bolyai' Research Scholarship of the HAS to B. Sellyei.

IODOBACTER PISCIPHILUM SP. NOV. IS ASSOCIATED WITH SKIN DAMAGE OF WILD AND CULTURED FISH

S. VILJAMAA-DIRKS[1]*, M. TIIROLA[2], S. HEINIKAINEN[1], H. KURONEN[1]

[1]*The Finnish Food Safety Authority Evira, Kuopio, Finland*
[2]*University of Jyväskylä, Jyväskylä, Finland*

During several years, unidentified bacterium belonging to the genus *Iodobacter* was isolated from the winter ulcers of wild bream as well as cultured salmonid fish in connection with saprolegniosis. Saprolegniosis is considered a secondary infection with water moulds, but in most cases the pathogenesis remains unsolved. Skin damage caused by trauma, hormonal imbalance or other forms of stress, or pathogens affecting the skin, alone or in combination with the other factors are suspected as the primary reason for saprolegniosis.
The *Iodobacter* sp. isolates from fish share the common features of the genus *Iodobacter* but have a lower growth temperature preference and lack the pigment production that is typical of the isolates of the type species *Iodobacter fluviatilis*. They prefer a low-nutrient medium and have recognisable spreading growth. Even in fish that have a heavy growth of *Iodobacter* sp. in the damaged skin areas, there is seldom any to be found in internal organs. Superficial skin damage is often contaminated by many other bacterial species that can interfere with the recognition of *Iodobacter* sp., especially if nutrient-rich media is applied.
We analysed 16 isolates of *Iodobacter* sp. by sequencing a 1500 bp gene segment of 16S rRNA and compared it with the bacterial library of closely related bacterial species. The isolates of *Iodobacter* sp. cluster together, apart from the recognised species *Iodobacter fluviatilis*, but show some variation between the isolates, also reflected in the macrorestriction analysis using X*ba*I enzyme and pulsed field gel electrophoresis. The genus *Iodobacter* is a group of environmental strains, and it is likely that *Iodobacter* sp. isolates from fish are of environmental origin. However, based on the molecular and biochemical analysis of our isolates, as well as the frequent isolation of this bacterium from fish skin and occasionally gills, we propose a new species *Iodobacter pisciphilum* sp. nov. to accommodate the unpigmented isolates collected from fish. The role of the pathogenesis of this bacterium in the skin pathologies of fish remains to be clarified.

AEROMONAS SOBRIA INFECTION IN FARMED MUD LOACH (*MISGURNUS MIZOLEPIS*) IN KOREA, A BACTERIOLOGICAL SURVEY

J.H. YU[1]*, B.H. KOO[2], D.H. KIM[2], D.W. KIM[3], S.W. PARK[2]

[1]*National Fishery Products Quality Management Service, Busan, Republic of Korea*
[2]*Kunsan National University, Gunsan, Republic of Korea*
[3]*Dong Gyeong Microorganism, Iksan, Republic of Korea*

A disease outbreak occurred in June 2013 among mud loach cultured in pond farms. Mortality rates reached up to 1.2% in the farm per day. Typical clinical sings were bleeding ulcer at the middle portion of head and haemorrhagic erosion of the operculum. Based on biochemical characteristics, the causative bacteria isolated from diseased fish belonged to the group of *Aeromonas sobria*. Histologically, liver showed hepatocellular vacuolar degeneration and congestion in sinusoids. The spleen exhibited necrotized splenocytes and haemorrhagic pulps. In the kidney, glomerular destruction, renal tubular necrosis and haemorrhage were observed. Experimental infection of normal mud loach with the isolate resulted in the development of clinical signs similar to those seen in the farm. The isolate expressed two haemolytic genes, aerolysin (*sob*) and haemolysin (*asa1*) genes. The results indicate that *A. sobria* is involved in the morbidity and mortality of the farmed mud loach.

A QUANTITATIVE PCR DEVELOPED FOR THE DETECION OF CARP EDEMA VIRUS AIDS IN THE DIAGNOSIS OF VIRAL DISEASES AND MORTALITIES IN KOI

M. ADAMEK[1]*, V. JUNG-SCHROERS[1], J. HELLMANN[1], F. TEITGE[1], S.M. BERGMANN[2], H. SCHÜTZE[2], D.W. KLEINGELD[3], K. WAY[4], D. STONE[4], M. RUNGE[5], D. STEINHAGEN[1]

[1]*Fish Disease Research Unit, University of Veterinary Medicine, Hannover, Germany*
[2]*Institute of Infectology, Friedrich-Loeffler-Institut, Greifswald, Germany*
[3]*Veterinary Task-Force, Lower Saxony State Office for Consumer Protection and Food Safety, Hannover, Germany*
[4]*Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, UK*
[5]*Food and Veterinary Institute, Lower Saxony State Office for Consumer Protection and Food Safety, Hannover, Germany*

The global trade of ornamental fish bears a great potential to spread viral diseases of fish. Carp edema virus (CEV), a pox virus has been known to cause koi sleepy disease (KSD) since the 1970's, however for a long time infections appeared to be limited to Japan. Recent communications are showing that the virus had been spreading worldwide. In spring 2014, high mortalities in koi associated with a clinical outbreak of KSD were noticed for the first time in Germany. However, the end-point PCR used for the detection of viral DNA confirmed the presence of the virus only in some samples.

To improve the diagnostic procedures for CEV, a quantitative PCR based on a dual labelled probe was developed. Trials were performed on gill and skin samples obtained from 26 morbid or dead koi that were or had been suffering from a disease with symptoms similar to KSD, and on samples from additional 7 koi without symptoms originating from the same tanks as the diseased fish.

For quantification of the CEV load in the infected tissue, a standard curve ranging from 10^0 to 10^7 copies was used. With this standard, the qPCR gave the amplification curve $y = -3.2499x + 41.706$ with a correlation $R^2 = 0.9988$ and an efficiency of 103.1%. The reliable detection limit of this qPCR was lower than 10 copies of CEV specific genes. The qPCR had a noticeably higher sensitivity than the previously described end point PCR. By screening 33 gill samples from koi, 19 fish were confirmed to be infected compared to 15 fish testing positive using the end-point PCR. In positive fish, the virus load ranged from 1 copy to 2046000 copies with a mean of 129982 and a median of 45 copies per 250 ng of isolated DNA.

The results confirmed that only some clinically affected individuals were positive for CEV and that these individuals had a very variable virus load. Therefore, the clinical symptoms observed in diseased koi could only be associated with a CEV infection in some cases.

DECENTRALISED MOLECULAR DIAGNOSTICS AND REMOTE DATA REPORTING FOR MANAGEMENT OF DISEASE IN GLOBAL AQUACULTURE

K.S. BATEMAN[1]*, M.J. POND[1], P. MUNDAY[2], O. GANDELMAN[2], G.D. STENTIFORD[1]

[1]*European Community Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK*
[2]*Epistem, Genedrive, Epistem Ltd., Manchester, UK*

Disease is widely acknowledged as the prominent bottle-neck to achieving global food security and poverty alleviation targets relating to aquaculture with annual losses exceeding US $6bn. High profile disease in $15bn shrimp industry include those caused by White Spot Syndrome Virus (WSSV), the bacterial pathogen implicated in Acute Hepatopancreatic Necrosis Disease (AHPND) and emergent pathogens such as *Enterocytozoon hepatopenaei*, implicated in Early Mortality Syndrome (EMS); these pathogens implicated in annual losses of $3bn per annum. Genedrive® is a small footprint molecular diagnostics platform capable of rapid, sensitive and specific detection of pathogens within an hour. It combines proprietary 'hybrid' thermal engine technology with bespoke consumable elements designed for detection of the pathogen/s of interest. An ultra-simple, 'single-button' operation allows for the operation of the equipment by un-skilled operatives, with minimal training. Developed for use in human pathogen diagnostics (currently tuberculosis testing in Africa), the technology has high potential for accurate detection of pathogens in other settings where rapid detection is required and where centralised laboratory infrastructure is poor. We are currently working to test and validate Genedrive® against gold standard diagnostics for WSSV and AHPND applied to penaeid shrimps. In addition, we are developing a bespoke smartphone app to interface with Genedrive® and to transmit field data to a centralised data repository for subsequent analysis. The formation of an accurate, low-cost diagnostic and integration with user-technology reporting of data has the potential to revolutionise disease management in global aquaculture and will contribute directly to poverty alleviation and global food security associated with aquaculture.

EVALUATION OF NON-DESTRUCTIVE MOLECULAR DIAGNOSTICS FOR THE DETECTION OF *NEOPARAMOEBA PERURANS*

J. DOWNES[1, 2]*, M. RIGBY[3], B. MAYNARD[3], R. TAYLOR[3], E. MACCARTHY[2], I. O'CONNOR[2], H. RODGER[4], N. RUANE[1], M. COOK[3]

[1]*Marine Institute, Galway, Ireland*
[2]*Galway/Mayo Institute of Technology, Galway, Ireland*
[3]*CSIRO, Hobart, Australia*
[4]*Vet Aqua international, Galway Ireland*

Amoebic gill disease (AGD), caused by *Neoparamoeba perurans*, has emerged in Europe as a significant problem for the Atlantic salmon farming industry and continues to be problematic in the Australian industry. Across the globe, gross gill score is the most widely used and practical method for determining AGD severity on farms and hence triggers for intervention. The use of molecular diagnostics for the early detection of the amoebae is commonly used in a number of countries such as Ireland, Norway and the Faroe Islands. As molecular diagnosis of AGD remains a high priority for much of the international salmon farming industry, there is a need to evaluate the suitability of currently available molecular assays and determine the most appropriate non-destructive sampling methodology.
The aims of this study were to assess a non-destructive sampling methodology (gill swabs) and to compare a range of currently available real-time PCR assays for the detection of *N. perurans* with regards to sensitivity and specificity. Furthermore a comparison of the non-destructive molecular diagnostics with traditional screening methods of gill scoring and histopathology was also undertaken. Correlations between gill scores and the real-time PCR results were performed and the suitability of each assay to detect *N. perurans* in water samples was also completed. A standardised protocol for non-destructive field sampling and molecular diagnosis of AGD will be presented.

VALIDATION OF THE SENSITIVITIES OF ONE-STEP AND TWO-STEP REVERSE-TRANSCRIPTION PCR METHODS FOR DETECTION OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) IVa ISOLATES FROM CULTURED OLIVE FLOUNDER (*PARALICHTHYS OLIVACEUS*) IN KOREA

H.J. KIM

Head Office, National Fishery Products Quality Management Service, Busan, South Korea

Viral hemorrhagic septicemia (VHS) is one of the most serious viral diseases of cultured olive flounder in Korea. This study validated the sensitivities of one-step and two-step reverse-transcription PCR (RT-PCR) methods based on the OIE (World Organization for Animal Health) diagnostic manual for the detection of VHSV type IVa isolates from olive flounder in Korea. VHSV type I was used as a positive control for the VHSV VN primer set listed in the OIE manual; the PCR products amplified from VHSV I appeared as strong gel bands of the target size, but the PCR products amplified from the VHSV IVa Korean isolate appeared as faint bands. Sequence comparison revealed that the VHSV VN forward primer was mismatched at 4 out of 24 nucleotide positions within the corresponding VHSV type IVa sequence, but differed only by a single nucleotide in the case of VHSV I. Therefore, the VHSV VN IVa primer set was designed specifically to fit the VHSV IVa sequence, and this was used in RT-PCR. The PCR products amplified from VHSV IVa appeared as strong bands of the target size when the VHSV VN IVa primer set was used, but the PCR products of VHSV I appeared as faint bands. PCR titration results showed that the sensitivity of the VHSV VN IVa primer set for VHSV IVa was 100,000-fold higher than that of the VHSV VN primer set when one-step RT-PCR was used, and that the sensitivity was >10,000-fold higher than that of the VHSV VN primer set when the two-step RT-PCR method was used with oligo dT+random hexamer. The sensitivity of the VHSV VN IVa primer set for VHSV IVa was found to be 10,000-fold higher than that of the VHSV VN primer set when the two-step RT-PCR method was used with the target primer set. Therefore, the RT-PCR methods that are used in pathogen-diagnosis laboratories must be validated according to the specificity of the primer set and the one-step or two-step RT-PCR method employed, using either the target primer set or oligo dT + random hexamer.

MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME OF FLIGHT, MALDI-TOF, MASS SPECTROMETRY FOR IDENTIFICATION OF FISH PATHOGENIC BACTERIA

E. JANSSON[1], E. ERIKSSON[1], E. SÄKER[1], I. DALSGAARD[2], B. NONNEMANN[2], I. ROOZENBURG[3], O. HAENEN[3]*

[1]*National Veterinary Institute, Uppsala Sweden*
[2]*National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark*
[3]*Central Veterinary Institute part of Wageningen UR, Lelystad, the Netherland*

Identification of bacteria has for long been performed by cultivation of samples on agar media, followed by biochemical characterization. During the last decades, molecular techniques as PCR have been widely used for a more rapid diagnosis. Recently, mass spectrometry by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) has been implemented as a new tool for bacterial a.o. pathogen identification at diagnostic laboratories. The bacterial colony on agar is the test sample, and with the MALDI-TOF technique, a unique spectrum is obtained, which is then compared with spectra in the developed database. The value of the MALDI-TOF technique is dependent on the extent and quality of the database. The database allows identification of a broad range of bacterial species, but is so far mainly focused on bacteria from human medicine. In the present collaborative study, fish disease laboratories in Denmark (DTU Vet), Netherlands (CVI) and Sweden (SVA) developed Main Spectra Projections (MSPs) for important fish pathogens from the genera *Aeromonas*, *Flavobacterium*, *Yersinia* and *Vibrio* for their own collaborative database. Both, bacterial isolates from routine bacterial diagnostics and isolates from the laboratories own collections have been tested by MALDI-TOF and compared with standard techniques for identification, as biochemical assays and 16S rRNA sequensing/PCR by use of the standard database and the new MSPs. *Flavobacterium psychrophilum*, *F. columnare*, *Vibrio anguillarum* and *Yersinia ruckeri* were all successfully identified to species level. Several serotypes of *V. anguillarum* and *Y. ruckeri* are known, as well as different serotypes and biotypes of *V. vulnificus* and *Y. ruckeri*. So far, differentiations to serotype or biotype for *V. anguillarum, V. vulnificus* or *Y. ruckeri* were not possible with MALDI-TOF, and need further study. The identification of different *Aeromonas* spp. by MALDI-TOF will also need further improvements, as the spectra showed too similar results among Aeromonas species for a correct identification. There are possible techniques available for subtyping bacteria by MALDI-TOF, and these are currently looked into for further studies.

FRANCISELLA NOATUNENSIS SUBSP. *ORIENTALIS* CAUSES MORTALITY IN MEXICAN TILAPIA (*OREOCHROMIS* SPP.)

C. ORTEGA[1]*, G. MANCERA[1], A. ROMERO[2, 3], R. ENRÍQUEZ[2], J. VÁSQUEZ[2], A. VARGAS[2, 3], S. MARTÍNEZ[1], R. FAJARDO[1], B. VALLADARES[1]

[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México*
[2] *Facultad de Ciencias Veterinarias, Universidad Austral de Chile; Valdivia Chile*
[3]*Centro FONDAP, Interdisciplinary Center for Aquaculture Research (INCAR), Chile*

Francisellosis in tilapia (*Oreochromis* spp.) is an emerging disease caused by the facultative intracellular bacteria *Francisella noatunensis* subsp. *orientalis* (FNO), which is present in several countries where fish farming of tilapia is an important economic activity. This work confirmed the existence of Francisellosis in fish from Mexico based on the study of a disease outbreak that occurred during the second half of 2012 in brood fish, with a mortality rate of approximately 40%. The disease was characterized by the presence of white nodules in different organs, mainly in spleen and kidney. Histologically, the lesions corresponded to the formation of granulomas. Based on DNA obtained from infected tissue and from a pure culture grown on cysteine heart agar medium supplemented with hemoglobin, FNO was initially confirmed by amplification and sequence analysis of the 16S genes and ITS of eubacteria and of the 16S rRNA gene specific of bacteria belonging to the genus Francisella. The phylogenetic analysis of these genes showed a close relationship with FNO sequences previously reported in tilapias from different countries; the subspecies was confirmed using the *iglC* gene as targeting sequence, yielding 100% sequence identity with the reference strains. The confirmation of the disease highlighted the importance of preventive actions to stop it from spreading throughout the country.

DEVELOPMENT OF A QUANTITATIVE SEMI-AUTOMATED SYSTEM, FOR HEPATOPANCREAS HISTOLOGY ASSESSMENT IN PENAEID SHRIMP, USING IMAGE ANALYSIS

P.F. SILVA[1]*, T. ERIKSEN[1], M. OWEN[1], D.V. LIGHTNER[2], B.L. NOBLE[2], F. CERVELLIONE[1], C. MCGURK[1]

[1]*Skretting Aquaculture Research Centre, Stavanger, Norway*
[2]*Aquaculture Pathology Laboratory, Department of Veterinary Sciences and Microbiology, University of Arizona, Tucson, USA*

Knowledge of the structural and functional alterations that occur in cells and tissue as part of adaptive or disease processes can provide a clearer understanding of the mechanisms underlying various tissue changes and pathologies. Thus, identification and characterisation of these changes are not only essential for understanding the plasticity of tissues but are necessary for the provision of definitive diagnosis of pathological states, for prediction of the course of the disease and for the guidance of therapeutic interventions.
Nowadays, the primary means of health assessment and disease diagnosis is performed through histology, and virtual microscopy is gaining momentum as an approach to supplement conventional histological evaluation methodologies. Also, it has long been recognised that the quantitative measurement of histological features can provide data, which can significantly improve the ability to make diagnostic-decisions in pathology. It allows description of the morphological changes in continuous numerical terms, which are likely to be more reproducible than ordinal grading. Furthermore, it assists the comparison process, as sets of numerical data are easier to manipulate and compare than a collection of images or descriptive notes. Moreover, statistical data can be more easily acquired from such quantitative measurements, making these methods particularly useful for hypothesis testing. Finally, such quantification of the data can also allow the detection of subtle changes not readily apparent to the unassisted observer. And at the same time, image analysis of digitised histological sections provides a practical means for quantifiable assessment of structural and functional changes in tissues.
With intensification of the Penaeid aquaculture industry, occurrences of disease have increased and are a major constraint to the profitability of shrimp aquaculture. Consequently, health assessment of the shrimp species has become a prime area of research, and histopathology innovative methodologies fundamental. Thus the primary purpose of this project is to focus on the development of a practical analytical methodology based on advanced image analysis, which in turn may be able to measure and characterise a range of features of shrimp's histology, in a quantitative manner. Preliminary quantitative data on the hepatopancreas histological structures will be illustrated.

A SINGLE PAIR OF DEGENERATED PRIMERS FOR THE DETECTION AND QUANTIFICATION OF A WIDE RANGE OF IPNV TYPES

D. VAZQUEZ*, C. LÓPEZ-VÁZQUEZ, J. M. CUTRÍN, C. P. DOPAZO

Instituto de Acuicultura, Universidad de Santiago de Compostela

Infectious pancreatic necrosis virus (IPNV) is the causative agent of an important disease in salmonid aquaculture. The disease produce major losses among first-feeding fry, and survivors can become life long carriers. Therefore, quick and reliable diagnosis is the most important tool to control the disease, and real time/quantitative PCR has become one of the best options. However, one of its drawbacks is the reduction of efficiency of the method due to the high diversity of this virus, what can frequently yield false negative results. All the methods published have been proved as reliable procedures for detection and quantification of one or a few genotypes at once. In the current study, we propose a degenerated pair of primers suitable for the detection and quantitation of 7 different serotypes of IPNV in the same reaction, using SYBR Green chemistry. The specificity of the primers was evaluated comparing their amplification efficiency over 7 different RNA standards, reaching efficiencies from 91.5% to 115%. The analytical specificity has been tested against a panel of more than 30 IPNV isolates, as well as other non-related viruses. The analytical sensitivity was assayed using both, RNA transcripts and crude virus as standards, and the results of quantification using the last one were compared with the plaque assay method. Finally the method was tested in parallel with traditional RT-PCR and cell culture isolation, using experimentally infected salmon fry. We are currently immersed in a process for the complete validation of the procedure, testing its reproducibility and robustness. Nevertheless, with the results obtained so far we can advance that the method will be a reliable procedure, not only for the detection but also for the quantification of IPNV both, *ex* vivo and in samples from clinically symptomatic and asymptomatic fish.

QUANTITATIVE FLOW CYTOMETRY ANALYSIS OF THE INTRACELLULAR EXPRESSION OF VP2 FROM IPNV

D. VAZQUEZ*, C. LÓPEZ-VÁZQUEZ, J.M. CUTRÍN, C.P. DOPAZO

Instituto de Acuicultura, Universidad de Santiago de Compostela

In the last decade flow cytometry has largely evolved, and its use for detection and quantification of virus-infected cells has added speed and automation for screening antiviral drugs and performing drug susceptibility assays. In the present study, we propose a novel procedure for the analysis of the intracellular viral gene expression and subsequent translation into viral proteins using a quantitative flow cytometry. The final aim of this study was the optimization and validation of a procedure for quantitation of the intracellular production of viral proteins, followed by quantitation of the viral mRNA per cell, using IPNV as a model. For the absolute measurement of the fluorescence, type IIIb standards (Quantum FITC-5 MESF kit, Bangs Lab) were employed, and the absolute values of fluorescence, expressed as Molecules of Equivalent Soluble Fluorochrome (MESF), were correlated with the RNA expression in each positive cell. For that purpose, the positive cells were sorted using a novel procedure validated by Hvartin et al (2014), and the RNA quantified by RT-qPCR. The linearity of the standards was evaluated, reaching in all cases values of $R^2 \geq 0.9936$. The reproducibility of the method was assayed, always yielding coefficients of variation lower than 3.04 % in the MESF values from positive samples, and between 1.8% and 8.3% for the MESF standard. The RNA quantification was performed using a specific RNA transcript to determine the copy number of preVP2 in each sample. We are currently working in the validation of the method for its use in different scenarios.

Reference: Hrvatin, S., Deng, F., O'Donnell, C.W., Gifford, D.K. & Melton, D.A. MARIS: Method for analyzing RNA following intracellular sorting. PLoS ONE 9, (2014).

INTER-LABORATORY PROFICIENCY TEST ON NOTIFIABLE FISH DISEASES: A TOOL TO STRENGTHEN DIAGNOSTIC CAPACITIES FOR VIRAL FISH DISEASES

N. VENDRAMIN*, S.S. MIKKELSEN, N.J. OLESEN

Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark

Targeted surveillance for severe fish diseases is a key tool for reducing the risk of disease spreading strengthening thereby the development of sustainable aquaculture. Listed diseases and related diagnostics methods in Europe are addressed in the European Council Directive 2006/88/EC and in its secondary legislation.
In order to assess the capacity of the Reference laboratories for fish diseases detect listed pathogens, to maintain their quality assurance system and to harmonize diagnostic tests globally, the European Reference laboratory (EURL) for fish diseases prepare, test and deliver yearly a Proficiency Test (PT). The PT, produced according to standard DS/EN ISO/IEC 17043, consists freeze-dried blind samples containing the causative agents of the listed exotic and non-exotic diseases (respectively EHN and VHS; IHN; ISA and KHVD.)
Representative samples of the different batch of ampoules are tested before shipment, in order to exclude cross contamination and guarantee homogeneity of the content; furthermore stability of the pathogens is checked after shipment.
Due to different diagnostic standards procedures for pathogen detection the PT is divided into two parts, PT 1 and PT2.

- PT1 targets VHSV, IHNV and EHNV. Participants are asked to isolate, identify and titrate the content of the ampoules using cell culture for isolation and immunochemical or biomolecular technique for identification.
- PT2 targets ISAV and KHV. Participants are asked to use PCR based techniques for pathogen identification.

Sequence analysis plays also an important role within the Proficiency test, where it is compulsory for EHNV discrimination from other ranaviruses, it is highly recommended for ISAV to differentiate pathogenic HPRΔ from non-pathogenic HPR0 strains and is encouraged for genotyping other pathogens.
Proficiency tests' results are anonymously compiled in a report where all participants can compare their performances both with the other laboratories and with the EURL assessing the efficacy of their laboratory procedures, their cell sensitivity and quality of the genetic sequences of the pathogens identified.
This activity started in 1996 and nowadays more than 40 laboratories partake it every year, an overview of the evolution of the success rate of the laboratories and the capacity of effectively diagnose listed diseases will be presented.

EFFECTS OF ATRAZINE ON NEOTROPICAL FISH KIDNEY, *PIARACTUS MESOPOTAMICUS*: A SUBCHRONICAL EXPOSITION AND RECOVERY ASSAYS

L.P. GIGLIO[1], M.C. DELCORSO[2], S.C.N. QUEIROZ[3], S. ARANA[4]*

[1]*School of Pharmacy (undergraduate research program), Campinas University, São Paulo Brazil*
[2]*Postgraduate Program in Cell and Structural Biology (master's degree), Campinas University, São Paulo, Brazil*
[3]*Laboratory of Environmental Microbiology, Brazilian Agricultural Research Corporation-EMBRAPA, São Paulo, Brazil*
[4]*Biology Institute, Campinas University, São Paulo, Brazil*

Environmental and human health risks that atrazine may cause justified its prohibition in European Union countries since 2005, but it is widely used in Brazil and often found above permitted levels (2.0 µg/l-1) in river basins. The aim of this study was to evaluate the effects of atrazine by toxicity and recovery assays on trunk kidney, the main target organ of this herbicide, on *Piaractus mesopotamicus* (pacu) to sublethal concentration (3.57 mg / l^{-1}) and non-lethal realistic concentration (3.0 µg/l^{-1}).
For the exposure assay 12 pacu fingerlings were kept for 30 days in glass tanks with test concentrations presented above, including the control group (0.0 µg/l^{-1}). The partial replacement of atrazine occurred every 72 hours with the renovation of 1/3 of the water volume. For the recovery assay, other tanks with 12 samples by group were maintained similarly, after 30 days gradually the contaminated water was substituted by clean water and the fingerlings were maintained for another 14 days under these conditions. At the end of each assay, kidney samples were obtained and processed to histopathological and ultrastructural analysis.
The kidney samples from all treatments showed *Myxobolus* sp. infection, which is common in Brazilian teleosts from natural environment or from fish farm, inducing degeneration of collecting tubule with massive reaction of melanomacrophages. Granulomas were also seen. The kidneys from sublethal exposition group showed also degenerative changes in proximal tubules, where no parasites or melanomacrophage reaction were identified. In nonlethal exposition group just few samples presented degenerative changes in proximal tubule cells. The recovery assay of sublethal group indicated that the degenerative alterations in proximal tubule were maintained. The recovery of nonlethal group showed no changes in proximal tubules. All groups from exposition and recovery assay presented renal tubular hyperplasia. These results indicated that the sublethal concentration of atrazine tested can induce serious damage to tubular renal function and those damages were not recovered when the fish were kept for 14 days in cleaner water and that the realistic non lethal concentration tested (found in Brazilian river basins) seems did not affect at all the function of the nephron.

Financial support: FAPESP: 2012/50197-2;
Master student supported by CAPES scholarship (Delcorso M.C.).

SCREENING FOR IMMUNOTOXIC POTENTIALS OF ENVIRONMENTAL CHEMICALS IN FISH: A LITERATURE REVIEW

L. BAUMANN*, K. REHBERGER, I. WERNER, B. HITZFELD, H. SEGNER

University of Berne, Centre for Fish and Wildlife Health, Berne, Switzerland
University of Berne, Centre for Fish and Wildlife Health, Berne, Switzerland
Swiss Centre for Applied Ecotoxicology, Dübendorf, Switzerland
Federal Office for the Environment, Bern, Switzerland
University of Berne, Centre for Fish and Wildlife Health, Berne, Switzerland

Numerous environmental chemicals, long known toxicants such as persistent organic pollutants as well as emerging contaminants like pharmaceuticals or nanoparticles, are known to modulate immune parameters of wildlife species. Thus, adverse consequences for the fitness of individuals including their capability to resist pathogen infections are very likely. Despite frequent field observations of impaired immunocompetence and increased disease incidence in contaminant-exposed wildlife populations, the potential relevance of immunotoxic effects for the ecological impact of chemicals is rarely considered in ecotoxicological risk assessment. Regarding the complexity and multifaceted nature of the immune system, it is unlikely that a single assay or parameter is sufficient for detecting immunotoxic potentials or effects of chemicals. For human toxicology, tiered testing frameworks are in place, which rely on a range of immunological tests and endpoints. In contrast, ecotoxicology does not possess an established inventory for the detection of chemical-induced immunomodulation and evaluation of the toxicological consequences. Therefore, the aim of the present study was to analyse the existing literature on immunotoxic effects of chemicals in fish to evaluate which immune assays and endpoints are most commonly responsive towards diverse chemicals and thus may have potential to serve as immunotoxicity markers in screening assays. 131 journal articles on immunotoxicity in fish were found in the databases of „pubmed“ and „sciencedirect“. Every article was analyzed in detail in the methods and the results part. Data were categorized concerning different categories, e.g. chemicals, fish species, exposure time, biomarkers or pathogens. The most frequently analysed parameters included phagocytic functions like respiratory burst or changes in expression of immune-related genes, predominately cytokines. The most extensively investigated classes of chemicals were hormones/EDCs, pesticides and nutrients/vitamins. Less frequently, different metals, pharmaceuticals and organic compounds were investigated. A single assay or parameter could not be determined to assess immunotoxic effects in fish. Instead, a comprehensive, tiered testing panel covering a range of assays and endpoints would be needed.

WATERBORNE METHYLMERCURY PRODUCES CHANGES IN ANTIOXIDANT AND IMMUNE STATUS IN THE GILTHEAD SEABREAM (*SPARUS AURATA* L.)

F.A. GUARDIOLA[1], E. CHAVES-POZO[2], J. MESEGUER[1], A. CUESTA[1]*, M.A. ESTEBAN[1]

[1]*University of Murcia, Murcia, Spain*
[2]*Instituto Español de Oceanografía (IEO), Puerto de Mazarrón, Murcia, Spain*

In the aquatic systems, the organisms are continuously exposed to several chemicals. Among them, mercury is an environmental contaminant that causes acute and chronic damage to multiple organs. In fish, practically all organic mercury is in the form of methylmercury (MeHg), which has been associated with animal and human health problems. In the present study we have evaluated the effects of waterborne exposure to sub-lethal concentrations of MeHg (10 μg L^{-1}) on the teleost fish gilthead seabream (*Sparus aurata*). Firstly, MeHg waterborne-exposed seabream specimens showed higher hepatosomatic index after 10 days, increased liver antioxidant enzyme activities after 2 days (superoxide dismutase and catalase), reduced serum biological antioxidant potential (BAP test) after 10 and 30 days and no effect on the levels of reactive oxygen metabolites (d-ROMs test). Regarding the immune response, serum complement was increased by MeHg waterborne-exposure after 30 days of treatment while the head-kidney leucocyte peroxidase and phagocytic activities were significantly increased after 10 and 30 days, respectively. This study describes, for the first time, the effects of waterborne MeHg exposure in the gilthead seabream immunity.

Financial support by grants AGL2011-30381-C03-01 and AGL2010-20801-C02-02 (MINECO and FEDER) and 04538/GERM/06 (Fundación Séneca, Spain) is gratefully acknowledged.

OOGENESIS IN MUSSELS (*MYTILUS GALLOPROVINCIALIS* LMK.) EXPOSED TO TARS: TIME-DEPENDENT HISTOPATHOLOGICAL EFFECTS

V. DOBAL-AMADOR[1]*, A. ALONSO-MARTÍNEZ[1], P. SUÁREZ-ALONSO[1], O. GARCÍA-MARTÍN[2], F. SAN JUAN-SERRANO[1]

[1]*University of Vigo, Vigo, Spain*
[2]*University of Santiago, Santiago, Spain*

PAHs are pollutants widely distributed in marine environment with toxic, mutagenic and carcinogenic effects on live organisms, which result in reproductive, metabolic and neoplastic disorders, but few studies have proved in invertebrates their causal relationship with these pathologies under laboratory conditions. In this study, we analyzed, under controlled laboratory conditions, the toxic potential of a tars mixture usually used in mussels farms and its histopathologic effects on gametogenesis of female mussels.
After calculation of median lethal concentration of this tars mixture (LC_{50} = 64.08 mg L^{-1}), mussels were exposed to the water-accommodated fraction of a sublethal concentration (60 mg L^{-1}) for seventeen days. Depending on exposure time, we observed histopathological changes in a progressive sequence of malignancy in germinal and connective tissue.
The disorders observed in germinal tissue were: atrophy of the gonadal follicles, arrest of oogenesis and vitellogenesis; pleomorphic oocytes with cytoplasm vacuolization and nuclear pyknosis, karyolysis and karyorrhexis; high phagocytic activity of degenerating oocytes by follicular (auxiliary) cells; fibrosis and hyalinization of follicular membranes which appeared occasionally broken allowing germinal cells to invade the connective storage tissue; proliferation and disposition of follicular cells in layers, obliterating the lumen follicular; and empty gonadal follicles lined by hyperchromatic germinal cells which could be considered as carcinoma in situ, precursor of neoplasias. The storage cells of connective tissue suffered hyperplasia and hypertrophy and the adipogranular cells showed also morphologic changes indicating a possible lipidosis. The number of oocytes released to water was drastically reduced and they showed severe anomalies as polar bodies highly fragmented, disorganized divisions and cell debris adhered to deformed oocytes.
These pathological disorders seems to indicate that the tars mixture tested causes among others effects, an endocrine disruption affecting the mussels reproduction, which could decrease both productivity of mussel farms and product quality.

IMMUNOLOGIC RESPONSES OF RAINBOW TROUT *ONCORHYNCHUS MYKISS* EXPOSED TO A CHRONIC POLLUTION OF HERBICIDE AND A VIRAL CHALLENGE WITH IHNV

C. DUPUY[1], J. CABON[1], L. LOUBOUTIN[1], T. MORIN[1], S. LE FLOCH[2], M. DANION[1]*

[1]*Anses, Plouzané, France*
[2]*Cedre, Brest, France*

Streams and ground water all around the world are contaminated by pesticides and several studies have already shown that contaminants can affect fish immune system and host resistance to pathogens. In fact, suppression of immune responses can lead to increase the sensibility to virus, the disease incidence and eventually the mortality of fish.
Main objective of this study was to evaluate the susceptibility of rainbow trout, *Oncorhynchus mykiss* to an experimental challenge with infectious hematopoietic necrosis virus (IHNv) after a chronic exposure to pendimethalin, an herbicide frequently used in agriculture and measured at high concentrations in rivers of several countries.
After 28 days exposure to 300 ng/L of pendimethalin, fish have been challenged by bath containing 10^4 $TCID_{50}$ mL^{-1} of IHNv. Four conditions were tested: 1) control, 2) contaminated by herbicide, 3) challenged with virus and 4) exposed to pendimethalin and IHNv. Mortalities were recorded during the 44 days post-infection (dpi) and organs were collected from dead fish for virological examination. Fish samples were made before and after the chemical contamination and 24h, 96h and 6 weeks after bath exposure to analyze specific and non-specific immune parameters. Lysozyme concentration, complement activity and the quantification of anti-IHNV antibodies were assessed in trout plasma. Expression of 8 genes implicated in immune system (C3-1 and C3-4, IFNγ, Il-β, TNFα1 and TNFα 2, TLR3 and β-defensin) were also followed in spleen.
Exposure to pendimethalin disturbs the immune system and the susceptibility of rainbow trout to viral challenge. In this study, pendimethalin seems have no direct impact on fish immunity but the chemical pollution modulates the immune response of fish in presence of IHNv. In fact, the number of fish which have set up a specific immune response is less in contaminated group by herbicide than in the control viral group. While β-defensin expression was down-regulated, IFNγ, Il-β and TLR3 expressions were up-regulated in fish exposed to pendimethalin and virus compared to those only challenged with virus. These results will be discussed and put in prospect to better understand the relationship between the different biological organization levels (molecular, cellular and individual).

EXPRESSED SEQUENCE TAQS (ESTS) ANALYSIS OF *NEOCARIDINA DENTICULATA DENTICULATA* FOLLOWING SHORT-TERM EXPOSURE TO COPPER

N.Y. KIM*, J.S. SEO, E.J. JEON, J.Y. HWANG, M.K. KWON, S.H. JUNG

Pathology Division, National Fisheries Research & Development Institute (NFRDI), Busan 619-705, Korea

Neocaridina denticulate denticulate exposed to copper dose dependant (1 - 100 ug Cu^{2+}/L for 24 h) and time dependant (1-24 h) were analyzed expressed sequence tags (ESTs). To analyze the transcriptome of the shrimp *Neocaridina denticulata denticulata*, we conducted EST analysis using seven cDNA libraries made from whole body. Redundant ESTs were assembled into overlapping contiguous sequences using the assembly program ICAtools. We found that the total 1,296 ESTs formed 185 clusters and 398 singletons, indicating that the overall redundancy of the library was 22%. Of the 1,393 clones, BLAST identified 1,278 clones (96.2%) as known genes; 115 clones (8.3%) did not match any previously described gene. Exposure to copper resulted in a significant effect the biological process, molecular function and cellular component. Based on the major functions of their encoded proteins, the identified clones were classified into 15 broad categories. Sequence analysis revealed the presence of microsatellite-containing genes that may be valuable for further gene mapping studies. This study contributes to the identification of numerous EST clones that can be applied to further clarifying the genetics and biomonitoring markers of shrimp.

TISSUE DAMAGES DUE TO *OSTREOPSIS OVATA* FUKUYO 1981 (DINOPHYCEAE) IN VERTEBRATE MARINE ORGANISMS

C. BERTUCCIO[2], D. PALOMBIERI[2], M.G. GIACOBBE[1]*, A. MANGANARO[3], G. LANTERI[4], F. MARINO[4]

[1]*Italian National Research Council, Institute for Coastal Marine Environment, Messina, Italy*
[2]*"FORINNOVAQUA" Formazione per l'innovazione tecnologica in acquacoltura, PON02_00667 – Codice Progetto PON02_00451_3362185*
[3]*University of Messina, Dept of Biological and Environmental Sciences, Messina, Italy*
[4]*University of Messina, Dept of Veterinary Medicine, Messina, Italy*

Dinoflagellates belonging to the genus *Ostreopsis* are common members of benthic microalgal communities in both tropical and temperate areas. Several *Ostreopsis* species produce palytoxin (PLTX) and its analogues, a class of highly potent toxins occurring in nature. The dangerous consequences of natural marine toxins can occur through phenomena of bioaccumulation along the food chain. PLTX targets membrane sodium-potassium pumps (Na^+/K^+- ATPase) responsible for maintaining ionic gradients. Since the last few years, blooms of *Ostreopsis ovata* have been occurring in the Mediterranean region with increasing frequency, intensity and distribution causing, sometimes, mortality of benthic organisms and human health problems. In order to improve our knowledge about the effects of *O. ovata* toxicity, directly on vertebrate marine organisms such as *Sparus aurata, Dicentrarchus labrax* and *Argyrosomus regius,* we performed an ecotoxicological screening by using a cultured strain of *O. ovata*, isolated from Isolabella (Ionian coast of Sicily) during a 2009-2010 study. Toxic effects on target tissues and organs and the survivability of the organisms exposed to *O. ovata* cells were investigated, in experimental conditions at two different temperatures (20 and 25 °C) and two different cell concentrations (500 cells ml^{-1} and 1.000 cells ml^{-1}). The results indicated that the effects of *O. ovata* on the marine organisms tested in this study depend on the concentration of *Ostreopsis* living cells used for the experimental contamination, the higher temperature amplifying the fish responses. In all exposed fishes, a mechanical damage to the gills and a consequent death by hypoxia were observed. Haemocyte aggregates surrounding both vegetative cells and temporary cysts of *O. ovata* were evidenced in histological observations of gills. Epithelial hyperplasia, secondary lamellar fusion and sometimes lamellar necrosis have also been documented.

CHLORINATED HYDROCARBON RESIDUES IN TISSUES OF FARMED AND WILD PIKE (*ESOX LUCIUS*)

R. PIETRZAK-FIEĆKO[1], E.TERECH-MAJEWSKA[1], M. MODZELEWSKA-KAPITUŁA[1], Z. ZAKĘŚ[2], A.K. SIWICKI[2]*

[1]*University of Warmia and Mazury in Olsztyn, Poland*
[2]*Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland*

Among chloroorganic insecticides, which cause much pathology in animals and humans, the most prevalent are γ-HCH and DDT. When accumulated in the fat tissue, cause teratogenic, mutagenic or carcinogenic effects. DDT may disrupt the intercellular connections, which may result in a loss of control over growth and differentiation of cells and has an impact on the blood cells and the immune, nervous and hormonal systems.
The aim of this study was to determine the content of selected chloroorganic insecticides residues in tissues of farmed and wild pike (*Esox lucius*).
Experimental material comprised of 11 farmed (aquaculture recilculating system RAS) and 13 wild pikes harvested in spring 2013 after spawning. Farmed and wild fish had similar body weight. Fish were originated from a lake situated in northern Poland. Chromatographic determination of γ-HCH, DDT, DDD and DDE was carried out with an Agilent Technologies 6890N.
In all tested samples the presence of γ-HCH, DDT and DDT metabolites: DDE, DDD were detected. The content of γ-HCH in tissues of wild pike was 2-fold higher (average 42.08 ng/g of fat) than in farmed pike. ΣDDT content was found also nearly 2-hold higher (average 355.06 ng/g of fat) in tissues of wild pike than in farmed ones. It should be noted that DDE constituted the highest percentage in ΣDDT.
The relatively high content of DDT in tissue of wild pike may indicate secondary contamination of the environment. However, in fish originated from RAS, chloroorganic insecticides were also present in all samples. Because fish is a major source of γ-HCH and ΣDDT in human diet, monitoring of these compounds in fish tissue, also those from RAS, is necessary.

PARALYTIC SHELLFISH TOXINS BY *ALEXANDRIUM* (DINOPHYCEAE) IN SEAWATER, CULTURES AND BIVALVE MOLLUSCS: CASE STUDY IN SICILY

M.G. GIACOBBE[1]*, A. COSTA[2], A. PENNA[3], E. RICCARDI[4], A. MILANDRI[4], E. GANGEMI[1], S. CAPPELLACCI[3]

[1]*Istituto per l'Ambiente Marino Costiero CNR, Messina, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy*
[3]*Università di Urbino, Pesaro, Italy*
[4]*Centro di Ricerche Marine, Cesenatico, Italy*

In the last years the presence of algal toxins in the aquatic environment and seafood has attracted great interest, with increasing geographical spread in marine waters of toxic dinoflagellates producing Harmful Algal Blooms. Marine biotoxins can accumulate in filter-feeders bivalve molluscs, such as the Mediterranean mussel *Mytilus galloprovincialis*, ingesting large quantities of plankton, and in organisms higher up in the food web. Birds and some fish can also be affected by PSP toxins and bivalve species exhibit a wide range of responses and sensitivities to them. Contaminated organisms may represent a potential for public health problems if toxins are transferred to humans through their consumption. HABs impact on aquaculture may give also economic losses due to temporary closures of unsafe shellfish harvest and marketing. Toxic blooms of dinoflagellates, such as several *Alexandrium* species, *A. minutum* and *A. catenella*, re-named in 2014 as *A. pacificum,* and occasional contamination of mussels, are reported in this study from a Mediterranean area, in the Ionian coast of Sicily (Syracuse Bay, 2011-2014) where shellfish farms are located. These species produce a suite of potent PSP neurotoxins (Paralytic Shellfish Poisoning), known collectively as "saxitoxins" which include pure saxitoxin (STX), neosaxitoxin (NSTX), gonyautoxins (GTX), and other derivatives, specifically binding the voltage-gated sodium channel, blocking the passage of nerve impulses with consequent paralysis. In the framework of our project on algal toxins, supported by the Italian Ministry of Health (Project RF-IZI-2008-1139874, Algal toxins contaminating water and fish products), the taxonomic identity of the two PSP-species was confirmed through the use of ribosomal markers (5.8S rDNA e ITS regions) in real time-PCR on field, seawater samples. Toxin profiles of the *Alexandrium* Ionian clones isolated from Sicily were obtained by HPLC chemical method and compared with the available data on PSP-toxins in mussels from our target location. Toxin profiles in the different samples revealed the presence of gonyautoxins (GTX1,4, GTX2,3, GTX5), C-group toxins (C1,2), and minor percentages of saxitoxins (STX, dcSTX).

PLASMA ALANINE AMINOTRANSFERASE (ALT) IN FARMED RAINBOW TROUT, *ONCORHYNCHUS MYKISS* (WALBAUM): PHYSIOLOGICAL VALUES AND FLUCTUATIONS IN DISEASES OR EXPOSURE TO TOXIC SUBSTANCES

J. ŘEHULKA

Department of Zoology, Silesian Museum, Opava, Czech Republic

Intensive salmonid culture exposes the fish to stress factors, affecting the basic physiological processes. An important role in fish health assessment is played by enzymes. Current knowledge and our experience shows that during the health screening of fish in intensive culture, an increased or reduced catalytic concentration of enzymes may highlight a factor responsible for the poor physiological state of the fish and contribute to supporting the diagnosis. The experimental fish was rainbow trout that were kept at an altitude of 652 m above sea level in flow-through type fibre-glass tanks 5×0.8×0.8 m in size at a stocking density of 50 kg m^{-3}, dissolved oxygen 8.4 – 13.5 mg L^{-1}, O_2 saturation of 77 – 98 % and at a photoperiod of 9 to 13 h : 11 to 15 h (light : dark). A direct non-parametric method was used to calculate physiological values in immature females with reproductive organs in the first stage of development (n = 360, mean weight 422 ± 144 g) and males with reproductive organs in the third stage of development (n = 28, mean weight 450 ± 111g). The physiological values determined by the lower (2.5 %) and upper (97.5 %) quantiles from raceway culture ranged between 0.01 and 0.66 μkat L^{-1} for females and between 0.08 and 0.54 μkat L^{-1} for males. Significantly ($p = 0.022$) higher values were recorded in males (0.25 ± 0.12 vs 0.19 ± 0.16 μkat L^{-1}). An increase in ALT was observed at the chronic stage of the VHS (1.16 ± 0.75 vs 0.58 ± 0.18 μkat L^{-1}) and in *Aeromonas* skin lesions (1.4 vs 0.11 μkat L^{-1}). In fish with the liver lipoid disease, the catalytic concentration of ALT was many times higher (0.35 to 10 vs 0.14 to 0.37 μkat L^{-1}). A reduced ALT activity (0.18 ± 0.06 vs 0.39 ± 0.14 μkat L^{-1}) was recorded in toxicological experiments with DEHP (bis(2-ethylhexyl)phthalate i.p.50 and 200 mg kg^{-1} 21 days) and with TCDD (2,3,7,8,-tetrachlorodibenzo-p-dioxine i.p. 2 μg kg^{-1} 21 days) (0.17 ± 0.06 vs 0.39 ± 0.14 μkat L^{-1}).

This work was financially supported by the Ministry of Culture of the Czech Republic through institutional financing of long-term conceptual development of the research institution (the Silesian Museum, MK000100595), internal grant of the Silesian Museum No. IGS 201506/2015.

THE OCCURRENCE OF *PETASIGER* METACERCARIAE (DIGENEA) IN AN UNUSUAL SITE, WITHIN THE LATERAL LINE SCALES OF CYPRINID FISHES

G. CECH[1]*, D.I. GIBSON[2], C. SZÉKELY[1], M. PAPP[3], P. DEÁK-PAULUS[3], L. JUHÁSZ[4], N. TÓTH[4], K. MOLNÁR[1]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*Department of Life Sciences, Natural History Museum, London SW7 5BD, UK*
[3]*National Food Chain Safety Office – Veterinary Diagnostic Directorate, Budapest, Hungary*
[4]*Department of Nature Conservation Zoology and Game Management, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary*

During a regular veterinary inspection of fishes from Lake Balaton, Hungary, echinostomatid metacercariae (Digenea), with collar spines characteristic of species of the genera *Petasiger* and *Paryphostomum*, were found in the lateral line scales of a roach (*Rutilus rutilus)*, an apparently unique site. In a subsequent examination of 586 fishes from 20 different species, similar infections were found in 11 species. The infection was virtually restricted to the lateral line scales, other scales being infected only incidentally. These encysted metacercariae had 27 collar spines, including eight larger angle spines and 19 smaller dorsal spines arranged in two rows. Two types of metacercarial cyst were found. The first type had three central dorsal spines that were larger than the remainder and tended to resemble the angle spines. The second type had all 19 dorsal spines of a similar size. ITS region and 28S rDNA (partial fragment) of the metacercaria were sequenced, and additionally adult specimens of *Petasiger phalacrocoracis, Petasiger exaeretus* and *Paryphostomum radiatum* collected from the gut of cormorant (*Phalacrocorax carbo*) were involved. ITS and 28S rDNA sequences of the second type of metacercaria (three samples) exhibited a 100% similarity to sequences of two adult *Petasiger phalacrocoracis* specimens collected from cormorants in Hungary and to *P. phalacrocoracis* deposited in the GenBank database. Interestingly, one sample of the second type metacercariae proved to be identical with two adult specimens of *Petasiger exaeretus* based on both ITS and 28S rDNA sequences. No morphological difference was observed between the metacercariae of the two *Petasiger* species. ITS sequences obtained from two metacercariae of the first type showed a 2.8–2.9 % difference from sequences of the second type of metacercaria and were not identical with any other *Petasiger* or *Paryphostomum* sequences. The 28S rDNA sequences supported these results with a ~1% difference from the second type metacercariae (*Petasiger phalacrocoracis)*. Based on these results, the second type metacercaria is considered to be a larval stage of *P. phalacrocoracis,* but the identity of the first type is uncertain. The unusual location of these metacercariae in the lateral line scales is discussed in relation to their transmission.

This study was supported by the grants KTIA-AIK-12-1-2013-0017, PD OTKA 108813 and OTKA 100132.

PISCIRICKETTSIA SALMONIS: MACROPHAGE APOPTOSIS REGULATION IN EXPERIMENTALLY INFECTED ATLANTIC SALMON (*SALMO SALAR*)

S. DÍAZ[1], M.E. ROJAS[1], M. GALLEGUILLOS[1], C. MATURANA[2], P.I. SMITH[3], P.A. SMITH[1]*

[1]*Facultad de Ciencias Veterinarias, Universidad de Chile, Santiago, Chile*
[2]*Virbac-Centrovet Ltda, Santiago, Chile*
[3]*Universidad del Desarrollo, Santiago, Chile*

Piscirickettsia salmonis is a facultative intracellular Gram-negative bacterium. This pathogen is the etiological agent of piscirickettsiosis, a systemic disease affecting a variety of teleost fish but particularly severe in salmonid fish reared in Chilean sea waters causing losses for at least US$ 100 million a year in this country. To improve the understanding of piscirickettsiosis pathogenesis, the *in vivo* modulation of macrophage apoptosis was studied in experimentally infected Atlantic salmon (*Salmo salar*). Post-smolts held in fresh water tanks were intraperitoneally inoculated with *P. salmonis*, which had been cultured in CHSE-214 cells. Control fish were sham-inoculated with disrupted uninfected CHSE-214 cells. Five fish, both from the infected and the control group, were randomly sampled at day 1, 5, 8, 20 and 40 post-inoculation (p.i.). Immediately after euthanasia, coelomic washings were obtained from each fish using a modification of the method described by Afonso *et al*. (1997). Macrophage populations of coelomic washings were analyzed by flow cytometry using the JC-1 cationic dye, as a mitochondrial membrane potential probe, to detect apoptotic cells. Comparisons of macrophage apoptosis percentages between infected and control fish showed no statistical differences at day 1, 20 and 40 p.i.. Nevertheless, macrophage apoptosis significantly decreased ($p < 0.05$) in the infected fish at 5 and 8 p.i. days. It has been previously reported that *P. salmonis* replicates inside macrophages (McCarthy *et al*. 2008) and the inhibition of the apoptosis in these leukocytes is consistent with this finding. Macrophage apoptosis modulation by *P. salmonis* could be important in the piscirickettsiosis pathogenesis as it would avoid an effective immune response against this bacterium and it would allow its multiplication and subsequent dissemination in infected fish.

Supported by Chilean Conicyt grants 1080692 (Fondecyt) and 21100325.

VIRUS REPLICATION AND HOST RESPONSE IN SALMONID GILL CELLS *IN VITRO*

T. HERATH*, M. WEIDMANN

University of Stirling, UK, FK9 4LA

The fish gill is continuously challenged by pathogens, toxicant and stressors from aqueous environment. The gill also plays an active role in pathogen entry and egress. The gill diseases in Atlantic salmon are a main concern, especially in terms of health and welfare in commercial aquaculture. The complex anatomy and the rich vasculature of the gill also make host-pathogen interaction studies carry out *in vivo,* difficult and sometime in-conclusive. In the present study, we used primary gill cells obtained from Rainbow trout, and continuous cells originated from Rainbow trout gills; RTgill-W1 to investigate innate immune competence of gill epithelium.
A protocol modified from Butler and Nowak (2004) was developed to isolate gill cells from and Rainbow trout (RTGL). The primary cells isolated form fish attached on to cell culture flasks rapidly, and formed monolayers with 100 % confluence within 7-8 days. The cell monolayers were primarily composed of epithelial cells and fibroblast cells. To study host-pathogen interaction in primary and secondary cells, the monolayers of cells were grown in 6-well tissue culture plates before stimulating with PMA and poly I:C or infecting with Infectious pancreatic necrosis virus (IPNV), salmonid alphavirus (SAV) and infectious salmon anaemia virus (ISA). The cells stimulated with PMA and poly I:C were sampled for RNA extraction 3 h, 6 h, 12 h and 24 h post exposure. The cells infected with virus were sampled for RNA extraction at day 1, 2, 3, and 5 post-infection. The virus replication and innate immune gene expression in primary and continuous gill cells were studied using quantitative real time PCR. Further, cell supernatants of virus infected cells were back titrated on TO cells to estimate 50 % tissue culture infective dose.
This study established preliminary data on host pathogen interaction in primary and continuous gill cells derived from trout in an *in vitro* platform. This system is expected to transform to create into a polarised gill epithelial cells system to study host response to a wide array of pathogens and also to test gill cell response to therapeutants, feed additives and vaccine antigens, addressing 3R in animal research.

METHODOLOGICAL ISSUES AFFECTING THE STUDY OF FISH PARASITES

Y. KVACH*, M. ONDRAČKOVÁ, P. JURAJDA

Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, 603 65 Brno, Czech Republic

Methods presently used in the study of fish parasites may have a significant influence on the results obtained. Problem areas are likely to include the sampling method used to catch host fish, and especially the degree of physical contact (manipulation), and how live fish are maintained prior to dissection.
In this study, we sampled Prussian carp (*Carassius gibelio*) and held them in a 1 m^3 basin outside. Twenty fish were dissected each day over the following six days. In addition, we compared the parasite community of white bream (*Blicca bjorkna*) sampled repeatedly from the same site using three common lentic sampling methods: electrofishing, beach seine and gill-nets.
Our results indicate changes in the ectoparasite community over time in the holding tanks, with the number of ciliates decreasing after four or five days and number of *Gyrodactylus* decreasing throughout the study. Level of physical manipulation by sampling gear (e.g. gill nets) was associated with absence of some common ectoparasite species, while host fish 'personality' (e.g. inquisitiveness) may not only increase the risk of exposure to higher numbers of endoparasites but also increase the fish's likelihood of capture. In order to obtain a representative assessment of a fish species' parasite community, therefore, these two important factors need to be taken into account; hence, we suggest using sampling methods that involve less physical contact (e.g. electrofishing) in future parasite community studies and that fish are dissected no later than three days after sampling.

This study was supported by a grant of the Czech Science Foundation (project P505/12/G112).

DEGENERATIVE ALTERATIONS IN TWO *MYXOBOLUS* SPECIES AND ITS HOST (*PROCHILODUS LINEATUS*) FROM MOGI-GUAÇU RIVER, BRAZIL: POSSIBLE ACTION OF XENOBIOTICS?

S. A. ZATTI[1], S. ARANA[2]*, A.A.M. MAIA[3], E.A. ADRIANO[1]

[1]*Universidade Federal de São Paulo, Diadema, SP, Brazil*
[2]*Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[3]*Faculdade de Zootecnica e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brazil*

Myxobolus porofilus and *Myxobolus lomi* (Myxosporea: Myxobolidae) are parasites of *Prochilodus lineatus,* an economically important South American teleost found in the Paraná-Paraguay and Paraiba do Sul rivers basins. This study provides ultrastructural analysis of *M. porofilus* and *M lomi* found infecting respectively gills and fins of *P. lineatus* caught in the Mogi Guaçu River, São Paulo State, Brazil, a river where the presence of agrochemicals has been frequently recorded the presence of agrochemicals. The prevalence was of 7.69% for *M. lomi* 15.38% for *M. porofilus*. Degenerative alterations were observed in gill epithelium of the fish and in the plasmodia and sporoblasts of both myxosporeans species. In *M. lomi* the plasmodia were surrounded by a capsule composed by layers of fibrocyte-like cells, with cellular projections joined to projections from other fibrocyte-like cells by desmosomes, and more externally typical fibroblast layers. Some granular leukocytes were seen interspersed among these layers. Abnormal electron translucent vacuoles were observed in the plasmodia periphery. In sporoblasts were observed myelin figures in the space between cytoplasmic membranes, and in immature spores was noted empty space (clefts) separating the cytoplasmic membranes of the cells, and vacuole formation in the intercellular region. In *M. porofilus* infecting fins the capsule of connective tissue was represented only by a loosely arranged collagen fibers and no granular leucocytes were seen. Vacuoles containing myelin figure were observed in the cytoplasm of the plasmodia and in the cells of immature spores. The possible influence of the inflammatory response was considered to explain the alterations observed in *M. lomi*. Nevertheless, the possibility of aquatic xenobiotics as inducers agents of the degenerative alterations observed in these two *Myxobolus* species and in their host was strongly considered since no intense host reaction was noted in *M. porofilus* as noted in *M. Lomi*. After the considerations above, the findings described here demand a deep investigation about the relationship of fish fibrocyte-like cell with other cells of the innate immune system to clarify their function in myxosporeans infections as well as in respect to the possible effects of xenobiotics on the abnormal development of the plasmodia of these parasites.

Master student supported by CAPES scholarship (S. A. Zatti). Study supported by CNPq (Proc. nº 477658/2010-5).

MORPHOLOGICAL AND HISTOPATHOLOGICAL CHARACTERIZATION OF HETEROPHYIDS (*PYGIDIOPSIS* & *HETEROPHYES*) ENCYSTED METACERCARIAE IN *MUGIL CEPHALUS* AT LAKE MANZALA, EGYPT

M.A. MAHMOUD*, M. ABDELSALAM, O.A. MAHDY

Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Sea mullet, *Mugil cephalus*, is one of the most important edible fish in Egypt. In this study, 120 specimens of sea mullet were collected from Lake Manzala, Egypt during February, April, July and November 2014. The muscles and internal organs of mullet were examined parasitologically and histopathologically for the presence of encysted metacercariae (EMC) of digenetic trematodes. The overall prevalence of encysted metacercariae in *Mugil cephalus* was 100% of all examined fish. EMC of *Pygidiopsis* & *Heterophyes* were found in different tissues of examined fish (head, trunk, tail, muscles, and internal organs). The identification of the parasites based on the morphological characters of EMC. The parasite cysts were varied in size, the presence of eye spots, and the shape of suckers, genital apparatus and excretory bladder. The histopathological examination revealed that, there were clear variations between the two cysts especially in the cyst shape and/ or tissue reactions against the cysts. In tissue sections, the cysts of *Pygidiopsis* sp. appeared rounded and with cyst wall while muscular oedema, hemorrhages and fibrosis were common. In case of *Heterophyes* sp., the cysts were elliptical and showed pericystic melanophores aggregation, oedema and atrophy of the skeletal muscle fibres. Eosinophilic granular cells infiltration in muscular tissue was a common finding in both cases. In internal organs, the histopathological examination revealed the presence of *Pygidiopsis* EMC in the abdominal fat, renal tissues and reproductive organs. This study concluded that, the heterophyid parasites could be identified in tissue sections depending on the morphological characteristics of EMC and their tissue reactions.

INVESTIGATION INTO *EX VIVO* MAINTENANCE AND SURVIVAL OF LARVAL SEA LICE, *LEPEOPHTHEIRUS SALMONIS* (COPEPODA, CALIGIDAE) USING TISSUE CULTURE SYSTEMS

H.C. MCDONALD[1], K.F. MUIR[1], J. IRELAND[1], S.J. MONAGHAN[1]*, C.M. MCNAIR[1], R.H. RICHARDS[1], D.P. KNOX[2], S. HAMILTON[2], T. KANELLOS[3] J.E. BRON[1]

[1]*University of Stirling, Stirling, UK*
[2]*Moredun Research Institute, Penicuik, UK*
[3]*Zoetis International Services, Paris, France*

The salmon louse, *Lepeophtheirus salmonis,* causes serious problems in salmonid aquaculture in the Northern Hemisphere, having huge economic impacts and welfare implications for the salmon industry. The use of integrated pest management strategies has provided some control; but a commercially viable vaccine to reduce infection would be highly advantageous. Current approaches for vaccine development involve using large numbers of fish; however, development of *in vitro* Atlantic salmon tissue models would assist research on host-parasite interactions and reduce research animal use. Fish scale models could provide the structural support and tissue stability needed for maintenance of sea lice but there are limited studies concerning their use.
Investigations into culturing scale-associated epithelial cells were undertaken, examining various parameters to optimise the conditions in order to maximise sea louse survival. The potential of the developed culture system to support different larval stages was also examined with regards to attachment of the motile infectious copepodid and survival of the sessile larval chalimus stage. The use of different agar constituents and conditions was investigated to determine whether maintenance of sessile larval chalimus could be achieved *ex vivo* and/or whether particular formulations would allow moulting of the larval stages. Lice were successfully maintained for > 10 days and moulting was observed in some cases. The attachment of infectious copepodids was examined through the use of scale-based agar in a variety of conditions. Toxicity studies to investigate the effect of cell culture products on sea lice highlighted difficulties in model development, and use of anaesthetics in harvesting tissue were found to affect the viability of attached lice stages. Development of a culture technique that allows maintenance of sea lice larvae could provide a platform for investigation of localised host-parasite interactions and with further optimisation could reduce the requirement for use of fish for production of sea lice and other aquatic ectoparasites.

MALFORMATION OF THE GILL FILAMENTS IN THE RUFFE [*GYMNOCEPHALUS CERNUA* (L.), PISCES] DUE TO INFECTION WITH METACERCARIAE

K. MOLNÁR[1], G. CECH[1]*, G. MAJOROS[2], CS. SZÉKELY[1]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*Faculty of Veterinary Science, Szent István University, Budapest, Hungary*

In parasitic surveys conducted on fishes of Lake Balaton and its tributaries in Hungary, infection with metacercariae supposedly belonging to the *Echinochasmus* genus (Trematoda: Echinostomatidae) was found in seven species of fishes belonging to five families. Malformations of the gill filaments caused by such infection were recorded in the ruffe *(Gymnocephalus cernua)*. The malformation was manifested as duplication of the filaments along about half their length. At the point where the filaments branched, a metacercaria incorporated in the cartilaginous gill rays of the filament was consistently found. Bifurcation was found in all of the ruffe specimens examined, andseveral other metacercarians causing only local distortions were found in the same fish specimens. In the other six infected fish species only local changes were found in the cartilage at the attachment sites of the metacercarians. By molecular methods, the ITS region of two metacercariae from ruffe was amplified and sequenced. The sequences showed 93.4% similarity to the ITS sequences of an *Echinochasmus* sp. sample (FJ756940), a cercaria collected from gravel snail (*Lithoglyphus naticoides*). It should be noted that a few other reference sequences originating from cercariae of the genus *Echinochasmus* are also available in GenBank, but those are sequences of the 28S rDNA. Other members of the family Echinostomatidae, such as *Echinostoma*, *Echinoparyphium*, *Paryphostomum* and *Petasiger* species, showed only a moderate similarity averaging between 82 and 84%.

This study was supported by the grants KTIA-AIK-12-1-2013-0017, PD OTKA 108813 and OTKA 100132.

ULTRASTRUCTURE OF *HENNEGUYA* A PARASITE OF *PSEUDOPLATYSTOMA PUNCTIFER* AND *LEIARIUS MARMORATUS* TAKEN FROM THE BRAZILIAN AMAZON

J. NALDONI[1]*, A.A.M. MAIA[2], E.A. ADRIANO[1]

[1]*Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Diadema, São Paulo, Brasil*
[2]*Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, São Paulo, Brasil*

The Amazon basin has an extraordinary range of fish fauna, with several species that are economically important for commercial and sport fishing and fishkeeping, and other species with fish farming potential. Among the fish that are economically important for extractive fishing and farming are species of the Pimelodidae family. The present study describes an ultrastructural analysis of *Henneguya* spp., a parasite of *Pseudoplatystoma punctifer* and *Leiarius marmoratus,* caught in the Rio Tapajós, Pará, Brazil. These siluriform pimelodids are known in Brazil as *jundiá* and *surubim,* respectively. *Henneguya* sp. 1 was found infecting the gill filaments of *P. punctifer*, and ultrastructural analysis showed the plasmodia was surrounded by connective tissue, with a few layers of fibroblast-like cells with cellular projections joined to projections from other fibroblast-like cells by desmosome junctions. In the host-parasite interface, this fibroblast capsule had a layer composed of fine granular material occupying the space between the plasmodia and the capsule. The plasmodial wall of the parasite was composed of a single membrane with numerous and extensive pinocytotic canals and projections towards the host tissue. There were numerous mitochondria just below the thin ectoplasm zone. Adjacent to this mitochondria layer there was a layer composed of generative cells and sporoblasts at different stages of development, with mature spores observed in the central zone. The binucleate sporoplasm had small, dark sporoplasmossomes and the filaments of the polar capsules were arranged in 10 turns. *Henneguya* sp. 2 was found infecting the gill filaments of *L. marmoratus* and ultrastructural analysis showed that the plasmodia was also surrounded by a thin capsule of fibroblast. There was a thin layer of electron dense granular material between this capsule and the wall of the plasmodia. The plasmodial wall was composed of a single membrane, which was linked to the ectoplasm by numerous pinocytic canals. Generative cells and sporoblasts at different developmental stages were found in the periphery of the plasmodia, and mature spores were found from the peripherical zone up as far as the deep zones of the plasmodia. This is the first study of myxozoans from *P. punctifer* and *L. marmoratus.*

Study supported by FAPESP (Proc. nº 2013/21374-6). Pos Doctor student supported by FAPESP scholarship (Proc. nº 2014/22700-7 - J. Naldoni).

MOLECULAR AND ULTRASTRUCTURAL DATA OF *MYXIDIUM* SP. PARASITE OF THE GALLBLADDER OF *CORYDORAS MELINI*, AN ORNAMENTAL FISH FROM THE BRAZILIAN AMAZON

P.M. DELGADO[1], J. NALDONI[3]*, M.R.M. SILVA[2], A.A.M. MAIA[2], E.A. ADRIANO[1, 3]

[1]*Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil*
[2]*Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, São Paulo, Brasil*
[3]*Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Diadema, São Paulo, Brasil*

Myxosporeans from specimens of bandit corydoras (*Corydoras melini* [Siluriformes: Callichthyidae]) taken from the Rio Negro in the municipality of Santa Isabel do Rio Negro, Amazonas, Brazil, were examined. The gallbladders of 23.3% of the 30 *C. melini* specimens examined were infected by an undescribed myxosporean of the genus *Myxidium*. The plasmodia of the parasite was tubular in shape. This tube resembled a spiral spring, with several turns within the gallbladder. The plasmodia had disporic sporoblasts that developed asynchronously. Mature myxospores were ellipsoidal from the frontal and lateral view, with slightly pointed ends. The surfaces of each valve had four to five longitudinal grooves. Spore dimensions were as follows: length 17.0±0.9 (16.1-17.9) µm and width 6.1±1.6 (4.5-7.7) µm. There were two polar capsules, one at either end of the spore, with length of 5.4±0.5 (4.9-5.9) µm and width of 3.4±0.6 (2.8-4.0) µm, and four to five polar filament turns. Ultrastructural analysis found that the wall of the plasmodia had a single membrane, with numerous long filiform expansions towards the bile fluid. Inside there were diasporic sporoblasts containing spores at different developmental stages. These sporoblasts were connected to each other through filiform expansions. The sequencing of the small subunit ribosomal RNA (ssrRNA) of the new *Myxidium* species resulted in 1790-bp, and this sequence did not match any of the Myxozoa available in GenBank. Phylogenetic analysis, with 30 species in the in-group and *Tetracapsuloides bryosalmonae* as the out-group, showed that *Myxidium* spp. formed two clades. The smaller clade was composed of four marine *Myxidium* species. The larger clade comprised marine and freshwater taxa of Myxidiidae (*Myxidium* and *Zschokkella* species plus *Cystodiscus melleni*) and Sphaeromyxidae (*Sphaeromyxa* spp.). *Myxidium* sp. appears in the large clade as a sister species to *Myxidium ceccarelli*, a parasite of the gillbladder of *Leporinus elongates*, a South American characiform from the Anastomidae family. This study is the first report of the *Myxidium* species found in freshwater ornamental fish in Brazil, and the first report of myxosporeans in *C. melini*.

Study supported by FAPESP (Proc. nº 2013/21374-6).
Doctoral student supported by FAPESP scholarship (Proc. nº 2013/14656-5 - P. M. Delgado).
Postdoctoral supported by FAPESP scholarship (Proc. nº 2014/22700-7 - J. Naldoni).

THE PARASITE COMMUNITY OF ENDEMIC *CHONDROSTOMA MEANDRENSE* (PISCES: CYPRINIDAE) FROM LAKE IŞIKLI, ÇIVRIL-DENIZLI, TURKEY

S. ÖZESEN ÇOLAK[1]*, E. SOYLU[2], M.P. SOYLU[2], M.S. UZMANOĞLU[2]

[1]*Fisheries Faculty, Istanbul University, Istanbul, Turkey*
[2]*Fisheries Department, Vocational School of Technical Sciences, Marmara University, Istanbul, Turkey*

Parasite community of *Chondrostoma meandrense* Elvira, 1987 in Lake Işıklı were studied. A total of 77 specimens of *C. meandrense*, 33 female and 44 male, mean (± SD) total length 20.8 ± 2.8 cm (range 17.0–27.8 cm) and mean (± SD) total weight 99.3 ± 46.9 g (range 42.5–211.7 g) were examined between July 2014 and March 2015 at monthly intervals. In five fish (6.5%) no parasites were found. In the other fish host were parasitized by at least one parasite species and the following parasite taxa recorded; *Ichthyophthirius multifiliis* (Ciliata), *Myxobulus* sp. (Sporozoa), *Dactylogyrus ergensi* (Monogenea), *Diplostomum* sp., *Tylodelphys clavata*, Digenea sp. (Digenea) *Bothriocephalus acheilognathi* (Cestoda), *Pomphorhynchus tereticollis* (Acanthocephala) *Argulus foliaceus*, *Lernea cyprinacea* (Crustacea) and *Unio* sp. Glochidia (Bivalvia). The most prevalent parasites were *Diplostomum* sp. (87.0%), *D. ergensi* (29.9%), *T. clavata* (18.2%) and *P. tereticollis* (15.9%).

P-187

HISTOPATHOLOGICAL FEATURES OF *DIDYMODICLINUS* SP. IN PSEUDOBRANCHES OF THE DUSKY GROUPER *EPINEPHELUS MARGINATUS* (OSTEICHTHYES: SERRANIDAE) FROM THE WESTERN MEDITERRANEAN SEA

M. POLINAS[1]*, S. MELE[1], O. REÑONES[2], E. ANTUOFERMO[1], P. MERELLA[1], F.E. MONTERO[3], F. PADROS[4]

[1]*Dipartimento di Medicina Veterinaria, Università di Sassari, Sassari, Italy*
[2]*Instituto Español de Oceanografía, Centre Oceanogràfic de les Balears, Palma de Mallorca, Spain*
[3]*Unidad de Zoología Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Paterna, Spain*
[4]*Fish Diseases Diagnostic Service. Facultat de Veterinària. Universitat Autònoma de Barcelona, Catalonia, Spain*

The dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Osteichthyes: Serranidae) is a widely distributed inhabitant of the Mediterranean rocky shores. Growing importance of this species in farming, artisanal fisheries and recreational scuba activities, requires attention to management of fish stocks. Grouper populations can be threatened by diseases caused by different pathogens, such as virus and parasites, e.g. didymozoid trematodes that can cause extensive damage to gills and other tissues.
The aim of this study is to describe the histological lesions and the inflammatory response caused by didymozoid trematodes in the pseudobranches of the dusky grouper from the western Mediterranean Sea.
Gill cavities of 115 dusky groupers, ranging from 44 to 104 cm total length, captured along the coast of Majorca Island (western Mediterranean Sea) from 1998 to 2014 were examined for didymozoids. Selected pseudobranches were formalin fixed, paraffin embedded and stained with hematoxilyn-eosin and Masson's trichrome. Parasites were identified using morphological methods.
Eighty groupers (70%) were infected by didymozoids. The parasitic capsules contained two yellowish convoluted worms, longer than 50 cm, which were assigned to the genus *Didymodiclinus*.
Histological examination showed the presence of multiple cystic structures in the pseudobranch epithelium, containing intact or degenerate adult trematodes and surrounded by a thick capsule of connective tissue. Inflammatory infiltrate was mainly organised around degenerate parasites, whereas intact ones lacked any sign of inflammation. Several thin vascularised septa were interposed between parasite coils, revealing an intimate contact between blood capillary endothelia and parasite body surface.
This study represents the first description of *Didymodiclinus* genus in pseudobranches of the dusky grouper. The close relationship observed between this trematode and the fish vascular system represents an important host-parasite interface that could play a particularly interesting functional role in didymozoid adaptation within tissues, for both nutritional or immunomodulatory purposes.

MORPHOLOGICAL CHARACTERIZATION OF GILL RESPONSE IN ATLANTIC SALMON (*SALMO SALAR* L.) INFESTED BY FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFER*A L.) GLOCHIDIA

M.I. QUIROGA*, A.M. DE AZEVEDO, C. VARELA, E. CORRAL, P. RONZA, A.P. LOSADA, R. BERMÚDEZ, R. AMARO, P. ONDINA

Facultad de Veterinaria, University of Santiago de Compostela, Lugo, Spain

Margaritifera margaritifera (L.) is a freshwater bivalve occurring in cool running waters of the Holarctic region. This naiad meets criteria of indicator, keystone, flagship and umbrella species and is thus an ideal target for the conservation of aquatic ecosystem. It is dramatically declining across Europe and considered one of the most imperiled freshwater mussels in the world. It is protected under the Habitats Directive, the Bern Convention and listed by IUCN as Endangered species. *M. margaritifera* has a complex life-cycle with parasitic larvae (glochidia) encysting on the gills of salmonids (Atlantic salmon, brown trout and sea trout), where they remain for several months before dropping off and settling into the river bed. The development of effective conservation programs depends of this parasitic stage, mostly achieved by the artificial infection of salmonids in captivity. However, there is limited information on the impact of glochidia on their hosts. The aim of this work was the morphopathological characterization of the response of Atlantic salmon (*Salmo salar* L.) to the presence of glochidia in gills during an experimental infection. Tissues were fixed in Bouin fluid, embedded in paraffin, and 3 µm-thick sections were stained with hematoxylin and eosin and PAS. Microscopically, gills contained multiple glochidia encysted at the top or along the length of filaments. Parasitized filaments were thickened, blunted, and often fused. Lamellae were extensively fused and obliterated, with proliferation of the epithelial cells, giving a smooth outline to parasitized filaments. Many glochidia were encysted between lamellae, associated with focal areas of epithelial hyperplasia and fusion. Occasional necrotic epithelial cells and pyknotic remnants were present within hyperplastic areas, which also showed localized increases in the number of mucous cells. Inflammatory infiltrates, sporadically surrounding glochidia, were present in hyperplastic areas and within the filament blood vessel below the lesions. Sometimes, degenerated-like glochidia were associated with a marked inflammatory reaction. These findings suggest that glochidia infestation produces branchitis of different severity degrees. In most severe cases, the infiltration by the host immune cells might be involved in the shedding of large numbers of the parasite throughout infestation.

Supported by "Consellería de Educación e Ordenación Universitaria", Xunta de Galicia (R2014/044), Spain and by the European Project Life + 09NAT/ES/00514.

IN VITRO AND IN VIVO DOWN-REGULATION OF MHC CLASS I IN THE COURSE OF CYHV-3 INFECTION

E. BORZYM, M. MATRAS, J. MAJ-PALUCH, M. STACHNIK, M. REICHERT*

Department of Fish Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland

The cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus has caused huge economic losses in common and koi carp culture industries worldwide since its emergence in the late 1990s. All known members of the family *Herpesviridae* show ability to establish life-long infections in immunocompetent hosts, which means that the host is incapable of getting rid of the virus completely. The detailed mechanism is very complex and not clear, but based on the reports from the study of mammalian herpesviruses, the inhibition of the host MHC class I antigen processing is considered as one of the key mechanisms of the so-called immune evasion strategy of the virus. In contrast to the mammalian system, the details of host-pathogen interaction in the case of CyHV-3 infection are much less understood. To get more knowledge on the immune evasion strategy employed by CyHV-3, we infected a CCB cell line as well as carp fry with CyHV-3 and measured the expression of host MHC class I in infected cells and internal organs of fish. A quantitative real-time PCR targeting the CyHV-3 thymidine kinase gene and a fragment of the MHC I sequence was developed. Glucokinase and beta-actin genes served as ~~an~~ internal controls. We found that the expression of the MHC I in infected cells was very significantly reduced compared to uninfected cells. This finding was true for both in vitro as well as in vivo system, as confirmed by statistical analysis. The highest differences in MHC class I expression in infected cells versus healthy ones were observed in the intestinum of infected carps, and the lowest in the brain. The differences in MHC I expression in the remaining organs were correlated with the virus load. In summary, we confirmed our hypothesis that CyHV-3 infection results in down-regulation of MHC I expression in infected cells; however, the detailed mechanism has to be examined.

SPARICOTYLE CHRYSOPHRII-INFECTED GILTHEAD SEA BREAM: EFFECTS ON GROWTH AND IMMUNE SYSTEM

G. RIGOS*, M. HENRY, C. NIKOLOUDAKI, C.S. TSIGENOPOULOS

Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Center for Marine Research, Aghios Kosmas, 16777 Hellinikon, Athens / Gournes 71500, Heraklion, Crete, Greece

Naïve gilthead sea bream were heavily infected by *Sparicotyle chrysophrii* two weeks following transfer of naturally-infected fish. The common signs of the disease were observed including anorexia, lethargy, anaemia and low mortalities. Growth reduction of infected fish was surprisingly not correlated with the parasitic load, possibly due to the unfavourable feeding ground for the parasite in small anaemic fish. Hemoglobin concentration and immunological parameters were also assessed at the end of the experiment. Overall, fish controlled the parasitic infection through an increased respiratory burst activity but *S. chrysophrii* suppressed the innate humoral components of the fish immune system making the fish potentially more sensitive to further opportunistic infections. The study underlined the complex host/parasite interactions involving the immune system of the host and the evasion mechanisms undertaken by the parasite. This was also confirmed through differences between recipient and control fish and correlations between those parameters and parasite load, fish weight and/or hemoglobin concentration.

INTERACTION BETWEEN *CALIGUS ROGERCRESSEYI* AND *PISCIRICKETTSIA SALMONIS* IN REGION X, CHILE, 2006-2007

S.S. BRAVO[1]*, M.T. SILVA[2]

Universidad Austral de Chile. Puerto Montt, Chile
Universidad San Sebastian, Puerto Montt, Chile

Piscirickettsia salmonis and the sea lice *Caligus*are the two most important pathological problems for the salmon industry in Chile. Outbreaks of *P. salmonis* have frequently been associated with severe infestations of sea lice in Chile since 1983, when outbreaks of *P. salmonis* were observed for the first time. In fact Lhorente et al. (2014) reported that *C. rogercresseyi* reduces the resistance of Atlantic salmon (*Salmo salar*) to *P. salmonis*. To elucidate the association between *C. rogercresseyi* and *P. salmonis*, data on farmed Atlantic salmon were collected and analysed from a field study carried out on three sites in the period 2006-2007. The farms were located in the same region of Chile, (42º40'S73º15'W). Abundance of *C. rogercresseyi* was correlated with the frequency of outbreaks of *P. salmonis* and the number of treatments used to control each pathogen in the period of the study. In that period emamectin benzoate (EMB) supplied in feed was the only treatment to control *C. rogercresseyi*. The data showed that site A applied two treatments to control *P. salmonis* and twelve treatments to control *C. rogercresseyi*; there were four and six treatments in site B and six and twelve treatments in site C respectively. These results are correlated with the abundance of *C. rogercresseyi* and with the environmental and geographical conditions of each site.

Reference
Lhorente, J.P., Gallardo, J.A., Villanueva, B., Carabaño, M.J., Neira R., 2014. Disease resistance in Atlantic salmon (Salmo salar): coinfection of the intracellular bacterial pathogen Piscirickettsia salmonis and the sea louse Caligus rogercresseyi. PloS ONE 9(4): e95397. doi:10.1371/journal.pone.0095397.

ROLE OF ECOLOGY, EVOLUTION AND IMMUNOLOGY FOR AQUATIC DISEASES IN RIVERINE LANDSCAPES: THE CASE OF PROLIFERATIVE KIDNEY DISEASE

T. WAHLI[1]*, N. STREPPARAVA[1], H. SCHMIDT-POSTHAUS[1], H. SEGNER[1], L. MARI[3], E. BERTUZZO[3], L. CARRARO[3], A. RINALDO[3], J. HOLLAND[4], C.J. SECOMBES[4], J. JOKELA[2], H. HARTIKAINEN[2]

[1]*Centre for Fish and Wildlife Health, University of Bern, Switzerland*
[2]*Department of Aquatic Ecology, EAWAG, Dübendorf, Switzerland*
[3]*Laboratory of Ecohydrology, EPFL, Lausanne, Switzerland*
[4]*School of Biological Sciences, University of Aberdeen, Aberdeen, UK*

The objective of this project is to develop insights into emerging aquatic diseases by integrating evolutionary, ecological and immunological approaches. More specifically, the project aims to study how environmental changes impact (i) maintenance, (ii) emergence and (iii) spread of the Proliferative Kidney Disease (PKD) of salmonids. The causative agent of this disease, *Tetracapsuloides bryosalmonae*, shows a complex multi-host life cycle, with freshwater bryozoans as invertebrate hosts and salmonids as vertebrate hosts. The innovation of this project is that it brings together demographic, genetic, immunological and ecological interactions of the disease agent and the host species across the entire parasite life-cycle. The understanding obtained from this integrated approach will enable the development of predictive models of the disease dynamics in riverine landscapes.

Three specific goals structure the project. Firstly, we study how the pathogen is maintained in fish and bryozoan populations. To this end, we investigate parasite transmission in relation to: host population structure and density, transmission routes and timing, parasite virulence and persistence and conduct molecular immunological characterisation of *T. bryosalmonae* - bryozoan interactions. Secondly, we investigate how susceptibility and infectivity drive disease emergence in changing environments that are affected by multiple environmental stressors, for example, by increasing temperatures, eutrophication and changes in habitat complexity. Thirdly, we address the epidemiological patterns that promote disease spread, integrating the genetic and ecological interactions between the pathogen and its fish and bryozoan hosts, and examine the pattern of local evolutionary adaptation that may further affect the spread of the disease.

Together the experimental and field studies provide the necessary parameterization for spatially explicit epidemiological models developed for dendritic river networks.

A HISTOLOGY-BASED FISH HEALTH ASSESSMENT OF FARMED SEA BASS (*DICENTRARCHUS LABRAX* L.)

A. SARAIVA[1, 2*], J. COSTA[2], J. SERRÃO[2], J.C. EIRAS [1, 2], C. CRUZ[1, 2]

[1]*Faculdade de Ciências, Porto, Portugal*
[2]*CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Porto, Portugal*

The health status of farmed sea bass (*Dicentrarchus labrax* L.) from a farm located in the north of Portugal was performed using a histological assessment based in the semi-quantitative system proposed by Bernet et al. (1999). Sea bass (n=30) were sacrificed via concussion. Gills, liver, kidney and intestine of each fish were fixed in 10% neutral buffered formalin for 48 hours and stored in 70% ethanol until processing. The tissues samples were processed for histological analysis using standard techniques in an automatic tissue processor, sectioned at 5 µm thickness and stained with haematoxylin and eosin (H&E). Histological changes are classified into five reactions patterns (circulatory, regressive, progressive, inflammatory and neoplastic). A degree of severity ranging from 1 (minimal severity) to 3 (marked severity) and a score value, ranging from 0 (unchanged) to 6 (severe/diffuse occurrence) were attributed to each lesion. These values were used to estimate the index for circulatory disturbance (IC), index for regressive changes (IR), index for progressive changes (IP), index for inflammation (II) and index for tumour (IT) for each organ and finally an organ´s index. Organ´s index values were used to classify the severity of histological response using the Van Dyk et al. (2009) classification system: Class I (index < 10) – normal tissue structure with slight histological alterations; Class II (index 10 – 25) – normal tissue structure with moderate histological alterations; Class III (index 26 -35) – pronounced histological alterations of organ; Class IV (index > 35) – severe histological alterations of organ. All these data will be presented and discussed in this communication.

This work was partially funded by the Project AQUAIMPROV (reference NORTE-07-0124-FEDER-000038), co-financed by the North Portugal Regional Operational Programme (ON.2 – O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF).

DEVELOPMENT OF A NON-INVASIVE PROCEDURE FOR DNA SAMPLING IN RAINBOW TROUT IN ACCORDANCE WITH THE 3Rs PRINCIPLE

S. COLUSSI, V. CAMPIA, M.V RIINA, T. SCANZIO, E. BURIOLI*, C. FOGLINI, M. PREARO, P.L. ACUTIS

Istituto Zooprofilattico of Piemonte, Liguria and Valle d'Aosta, Turin, Italy

The care and use of live animals for scientific purposes is governed by internationally established principles of replacement, reduction and refinement already known as 3Rs principle, reported for the first time by Russel and Burch (1959). These principles should be implemented following a strict hierarchy starting with replacement of animals with alternative methods such as *in vitro* methods. When it is not possible, the number of animals used should be reduced as much as possible to maintain statistical significance, while refinement should be based on non-invasive methods and on the reduction of manipulation-related stress.
Different fish species are gaining increasingly higher importance worldwide in biological, molecular and biochemical research. Their use in these studies, even if after years of debate about their sentience, is ruled by specific laws applied to the vertebrates (at European level Directive 2010/63).In accordance with the refinement principle, we improved buccal swab as non-invasive procedure to get DNA from rainbow trout. To optimized DNA extraction, we compared at 72 hours, different storage systems. Buccal swabs, collected from 30 fish, were subdivided in three groups: 30 dry swabs, 30 preserved in PBS and 30 in ethanol, all transported and stored at 4°C. DNA extraction was carried out directly from dry swabs, while PBS and ethanol swabs were centrifuged to obtain a cell pellet, usable also for further investigations, in contrast to dry swab. Only DNA got from PBS and ethanol swab had good quality and permitted amplification of nuclear targets. Ethanol is reported to avoid biotic degradation stopping bacteria proliferation and to inactivate endogenous DNases. DNA degradation seems in fact to have occurred in dry swabs, probably related to microbial population hosted in fish buccal cavity. DNA damages have already been reported in freshwater fish cells stored in PBS. This may be related with the low osmolarity of PBS compared to fish cells causing injury to the nucleus. Nevertheless DNA amplification for dry swabs was seen for mitochondrial targets, often selected for their stability for forensic purposes. Considering these results altogether, we suggest ethanol as the best choice for buccal swab sampling in genetic studies.

ANISAKIS SPP. IN ANCHOVIES CAUGHT OFF THE LIGURIAN COAST

L. SERRACCA[1], R. BATTISTINI[1], I. ROSSINI[1], A. TERAROLLI[1], M. CORSI[1], M. PREARO[2]*, E.A.V. BURIOLI[2], M. RIGHETTI[2], C. ERCOLINI[1]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, La Spezia, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Laboratorio di Ittiopatologia, Torino, Italy*

Human anisakiasis is a seafoodborne parasitic zoonoses caused by the accidental ingestion of anisakid nematode third-stage present in various fishery products. Initial manifestations of infection include general gastrointestinal symptoms while subsequent sensitization to *Anisakis*-derived allergens can raise the risk of allergic exacerbation on secondary exposure. Anisakiasis is particularly widespread in those countries where the use of raw seafood or not properly treated is made. In Italy and particularly in coastal areas, the local gastronomy involves the use of processed fish products with technologies not suitable for the devitalization of the parasite. The aim of this study was to investigate the occurrence of *Anisakis* larvae in anchovies caught in the Mediterranean Sea and to perform a biomolecular characterization of the L3 larvae isolated to identify the larvae at the species level. From April to September 2012 a total of 1,050 anchovies (*E. encrasicolus*) were analysed. The samples were transported within 24 h and kept refrigerated until analysis. Fishes were subjected to a visual inspection and/or by stereomicroscope; viscera and fillets were pressed to 1–2mm thickness to observe the larvae more easily. The flattened fillets or viscera were bagged and then observed under a stereomicroscope. Larvae were isolated and identified at the genus level according to morphological characters. The larvae morphologically identified as belonging to the genus *Anisakis* underwent molecular characterization by PCR-based restriction fragment length polymorphism (PCR-RFLP) to identify the species. A total of eight *Anisakis* spp. larvae were isolated and they were all viable and found in the visceral mass but not in the musculature. All infected fish samples harboured only a single larva. All eight larvae morphologically referred to the genus *Anisakis* showed the restriction profiles corresponding to *A. pegreffii.* The values of intensity and average abundance were respectively equal to 1 and at 0.0076. Anchovies represent a large portion of Italian fisheries production and human seafood consumption; however, information on *Anisakis* spp. infection from Italian waters is limited. According to these data, the risk of acquiring anisakiasis from the consumption of anchovies fished in this area seems to be very low.

BACTERIAN CONTAMINATION OF RED MULLET: *MULLUS BARBATUS* (LINNAEUS, 1758) FROM WESTERN ALGERIA

HEBBAR CH[1, 2]*, D. MARZOUG[2], Z. BOUTIBA[2]

[1]*Laboratoire d'Ingénierie en Sécurité Industrielle et Développement Durable (LISIDD)/ IMSI/ Université Oran 2 Mohamed Ben Ahmed*
[2]*Laboratoire Réseau de Surveillance Environnementale (LRSE) /Université Oran1 Ahmed Ben Bella*

Fish products are a reservoir of infectious agents (viruses, fungi, bacteria and parasites) naturally present in the aquatic environment or introduced through human activity.
This study was performed to evaluate bacterial contamination of a teleost benthic fish species, a red mullet. *Mullus barbatus* (Linnaeus, 1758) has an important commercial interest in the Algerian coasts of the western Mediterranean.
A total of 30 specimens were collected from Oran coast (western Algeria) in 2015. The methodology for the detection of microorganisms was carried out on the basis of international standardization (ISO), standards established by Ministerial Decree of 24 January 1998 on the microbiological specifications of foodstuffs.
Search of aerobic microorganisms at 30 ° C may indicate spoilage. Fecal coliforms can detect a native of fecal contamination. *Staphylococcus aureus* indicated human contamination. *Salmonella* is rarely present in fishery products and the presence indicates a non respect of the hygiene. The study revealed the absence of *Vibrio*, *Staphylococcus aureus* or *Pseudomonas*. This result is an indicator of bacteriological contamination and showed that samples markets were contaminated with potential pathogenic microorganisms. All these bacterial groups can detect a deficiency in the application of good hygiene practices.

MONITORING OF ORNAMENTAL FISH IMPORTS FOR POTENTIAL ZOONOTIC AND ANTIBIOTIC RESISTANT BACTERIA, AND TRANSPORT WATER FOR RESIDUES OF THERAPEUTICS IN THE NETHERLANDS

O. HAENEN[1]*, I. ROOZENBURG[1], T. ZUIDEMA[2], K. RIEPEMA[1], N. TAFRO[3], L. STOLKER[2]

[1]*Central Veterinary Institute, part of Wageningen UR, Lelystad, the Netherlands*
[2]*RIKILT, part of Wageningen UR, Wageningen, the Netherlands*
[3]*NVWA, Schiphol, the Netherlands*

In global ornamental fish trade, yearly, ~3000 deliveries of ornamental fish are imported into the Netherlands from 40 countries worldwide. These fish may carry potential zoonotic bacteria, and multi-resistant bacteria. Moreover, fish transport water may contain residues of therapeutics.
In this study, the Veterinary Authority NVWA sent 50 batches of various imported freshwater ornamental fish species, mainly from Asia and South America, in their original bag, directly from Schiphol Airport, for analysis. Transport water per batch was sampled for residues of therapeutics, from 2 fish per batch bacteria were isolated from skin and internal organs, and a liver smear was stained Ziehl Neelsen (ZN).
At RIKILT, the water samples were analyzed for residues of antibiotics (tetracyclines, sulfonamides, macrolides, fluoroquinolones, β-lactams, aminoglycosides, nitrofuranes, chloramphenicol and (leuco)malachite green). Forty-nine of the fifty water samples were found positive for one or more of the antibiotics in concentrations ranging from 0.02 to 10000 µg/kg.
At CVI, from multi-bacterial cultures, strains of *Aeromonas* spp. (59x) were identified by MALDI-TOF, and sensitivities were tested against tetracycline, flumequine, trimethoprim + sulphadizine, neomycine, florfenicol, and nitrofurantoin. From 59 strains of *Aeromonas* spp. 85% were resistant against tetracycline, 52% against flumequine, 31% against trimethoprim + sulphadizine, 34% against neomycine, 9% against florfenicol, and 17% against nitrofurantoin. Concerning potential fish zoonotic bacteria, one fish batch was strongly ZN-positive, suggesting fish TBC.
In our study, imported warm water ornamental freshwater fish mostly carried various resistant opportunistic *Aeromonas* spp., but seldom mycobacteria, potentially zoonotic. Often the transport water contained residues of antibiotics, authorized and non-authorized. These fish imports may pose a small to medium risk to man, at direct contact with fish and fish water. Hygienic measures should always be in place.

We acknowledge NVWA, who subsidized this project.

ANISAKIS PEGREFFII (NEMATODA, ANISAKIDAE) TOTAL ANTIGEN EXERTS OXIDATIVE STRESS AND MODULATE THE EXPRESSION OF BIOMOLECULAR MARKERS RELATED TO INFLAMMATION AND CANCER IN HUMAN CELL LINE

C.M. MESSINA[1], A. SANTULLI[1], M. BOBAN[2], S. ORHANOVIĆ[2], I. BUŠELIĆ[3], I. MLADINEO[3]*

[1]*University of Palermo, Dept DiSTeM, Laboratory of Marine Biochemistry and Ecotoxicology, Trapani, Italy*
[2]*Faculty of Natural Sciences, University of Split, Split, Croatia*
[3]*Institute of Oceanography & Fisheries, Split, Croatia*

Humans can become accidental host after consumption of raw or thermally inadequately treated fishery products that are contaminated by alive third-stage (L3) *Anisakis* larvae. Different types of anisakiasis have been recognised; gastric, intestinal, ectopic or gastroallergic anisakiasis, in hypersensitized patients. In countries where both the prevalence of human cases and general awareness of this zoonosis is high, there have been described rare cases of bowel carcinomas associated to the isolated larvae. It is still unclear if two aetiologies, e.g. anisakiasis and carcinoma are related or accidental incidences, but it is indicative that in all reported cases, neoplasia and embedded larvae shared the common site affected by chronic inflammation. In line with this, the aim of our study was to test the effect of *A. pegreffii* total antigen (ATA) on human normal fibroblast cell lines HS-68 and subsequently measure the level of oxidative stress (ROS), inflammatory and carcinogenic markers (Hsp70, p53, c-fos, c-jun and TNF). Two types of ATA were used; the first obtained from parasite excretory/ secretory production (ES), and the second obtained by whole-nematode ethanol extraction (CE). Preliminary results evidenced that cells have undergone a progressive reduction in vitality after ATA stimulation in dose and time-dependent matter, but the effect differed in respect to the antigen product applied. Similarly, ROS was significantly increased compared to control cell line, and highly correlated to cell vitality. Expression of p53, c-foc and c-jun, quantified by q-PCR was congruent to immunoblotting detection of Hsp70 and TNF at protein level, all being significantly upregulated in affected cells. This suggests that *Anisakis* total antigen products can indeed trigger two pathways; the first one is oxidative stress pathway characterized by inflammatory and apoptotic pattern in cells subjected to *Anisakis* excretory/ secretory products, while the other is more toxic effect characterized by induction of carcinogenic markers and blockage of inflammation and apoptosis in cells subjected to parasite crude extract.

The study has been financed by Croatian National Scientific Fund HRZZ Angel (GA# 5576) and European Social Fund GA# HR.3.2.01-0276.

THE ADJUVANT EFFECT OF LOW FREQUENCY ULTRASOUND WHEN APPLIED WITH AN INACTIVATED *AEROMONAS SALMONICIDA* VACCINE TO RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

C. COBO[1]*, R. JUNG[2], G. KOTTERBA[3], S. BERGMANN[3], R. ARIAV[4], P. ILARDI[5], K. THOMPSON[6], W. KLOAS[1], K. KNOPF[1]

[1]*Leibniz-Institut für Gewässerökologie und Binnenfischerei, Müggelseedamm 310, 12587 Berlin*
[2]*BANDELIN electronic GmbH & Co KG, Heinrichstraße 3-4, 12207 Berlin*
[3]*Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems*
[4]*Aqua-Vet Technologies Ltd., 45 Hasuca St. N. Habaron, Zichorn Ya'akov 30900, Israel*
[5]*Veterquímica S.A. Research and Development Laboratory, Santiago, Chile.*
[6]*Moredun Research Institute, Pentlands Science Park/Bush Loan, Penicuik, Edinburgh*

The adjuvant effect of low intensity low frequency sonophoresis (LFS) was tested in rainbow trout using an *Aeromonas salmonicida* bacterin vaccine administered by immersion vaccination using LFS at 37 kHz. Quantitative PCR was used to measure bacterial DNA in vaccinated fish up to 35 days post-vaccination, while RT-qPCR was used to assess gene expression during the early and late immune response post-vaccination. Results showed that antigen uptake in the gills was significantly higher in the group exposed to low intensity LFS compared to the other two vaccination groups 15 min post-vaccination, but this initially high uptake did not persist over the rest of the experiment. In the kidney, by comparison, the vast majority of the samples analysed did not show the presence or persistence of the bacterin. Showing that the route of vaccine uptake using the *A. salmonicida* bacterin, does not influence the persistence of the bacterin in the gills or the kidney. On the other hand, LFS induced a higher inflammatory response and T-helper cell activation, characterized by a significant up-regulation of interleukin (IL) 8, IL1ß and CD4, respectively. The expression of IgM, IgT and IgD was up-regulated in gills (being significant for IgM and IgD), but not in the spleen and kidney of the sonicated group. Conversely, IgM was up-regulated in the spleen of the non-sonicated groups, but not in the sonicated group. This highlights that the inflammatory response caused by ultrasound can boost mucosal immune responses, so that ultrasound shows an adjuvant-like effect.

ASSESSMENT OF VACCINE STRAIN COMPARED TO THE VIRULENCE OF DIFFERENT ISOLATES OF *AEROMONAS HYDROPHILA* IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

A. KUBILAY[1]*, S. ÇIFTÇI[1], P. YıLDıRıM[1], E. GÜMÜŞ[2]

[1]*Department of Aquaculture, Faculty of Fisheries, Suleyman Demirel University, 32260 Isparta, Turkey*
[2]*Department of Aquaculture, Faculty of Fisheries, Akdeniz University, 07058 Antalya, Turkey*

The purpose of this study was to determine the vaccine strains of pathogenic *Aeromonas hydrophila*. The bacteria are ubiquitous and heterogeneous organisms. *A. hydrophila* infection is a systemic disease that leads to the motile Aeromonas septicemia. Particularly in rainbow trout culture is considered major economic problem with other secondary infection. At present, there is not commercially a valiable vaccine. The lack of commercial vaccines can be relation of the high heterogeneity of this bacterium. In this study were determined virulence of ten *A. hydrophila* isolates from diseased rainbow trout (8 isolates), carp (1 isolate) and gold fish (1 isolate). The all isolates were biochemically smilar. A total of 240 rainbow trout were used for testing the degree of virulence of selected isolates. The strains were adjusted to 1.2×10^8 cfu/ml by colony count. 20 fish were injected to the pathogen isolates, 20 fish were injected using the same procedure with PBS as control, and other 20 fish were held untreated control. Mortality was monitored daily for 21 days. Internal organs of all dead fish were examined to confirm *A. hydrophila* infection by re-isolattion inoculated strains. The most virulent strain was observed with 60% mortality AHS (isolate of diseased carp) and 45% mortality AH1 (isolate of diseased rainbow trout). Some strains were considered as non-virulent, because of kill fish at 10^8 cfu/ml. The rainbow trout isolates were less virulent than those of carp. Differences in relative virulence of strains of *A.hydrophila* are the most significance form of heterogencity for fish vaccine development.

IN VITRO ANTIBACTERIAL ACTIVITIES OF POMEGRANATE (*PUNICA GRANATUM* L.) AND GRAPE (*VITIS VINIFERA*) VINEGAR AGAINST BACTERIAL FISH PATHOGENS

A. KUBILAY[1]*, S. METIN[1], P. YıLDıRıM[1], H.N. BUDAK[2], G. ULUKÖY[3], E. GÜMÜŞ[4], Z.B. GÜZEL-SEYDIM[2]

[1]*Department of Aquaculture, Faculty of Fisheries, Suleyman Demirel University, 32260 Isparta, Turkey*
[2]*Department of Food Engineering, Suleyman Demirel University, Çünür 32260 Isparta, Turkey*
[3]*Department of Aquaculture, Faculty of Fisheries, Muğla Sıtkı Koçman University, Muğla, Turkey*
[4]*Department of Aquaculture, Faculty of Fisheries, Akdeniz University, 07058 Antalya, Turkey*

The aim of this study was to evaluate the antibacterial activity of pomegranate and grape vinegar against bacterial fish pathogens. For this purpose two vinegars from pomegranate and grape were prepared using surface culture method. Their antibacterial activity was determined by using agar well diffusion assays. The vinegars were tested against fish pathogens; *Vibrio anguillarum, Yersinia ruckeri, Aeromonas hydrophila* (ATCC 7966), Staphylococcus *epidermidis, Lactococcus garvieae* and *Lactococcus garvieae* (ATCC 43921). Pomegranate vinegar showed that remarkable antibacterial activity against all the tested pathogenic bacteria. The zone diameter of inhibition of pomegranate vinegar was determined against *V. anguillarum* (21.5 mm), *S. epidermidis* (19.5mm), *A. hydrophila* (ATCC 7966) (18 mm), *Y. ruckeri* (17 mm), *Lactococcus garvieae* (10.5 mm) and *Lactococcus garviae* (ATCC 43921) (13.5 mm), respectively. Grape vinegar displayed moderate antibacterial activity against all the tested bacteria with a diameter of inhibition zone ranging between 8.5 mm and 15 mm. Antibacterial and immunostimulant features of these vinegars are known to be therapeutic for human health in many diseases. The present study showed that they are also being found to be effective against fish pathogens. The results confirmed the possible use of vinegars as a source of antimicrobial agents, which could be used in aquaculture for the control of bacterial infections.

IN VITRO ANTIBACTERIAL ACTIVITIES OF *SATUREJA CUNEIFOLIA* TEN. ON BACTERIAL FISH PATHOGENS

S. METIN, B.I. DIDINEN*, P. YILDIRIM, A. KUBILAY

Department of Aquaculture, Faculty of Fisheries, Suleyman Demirel University, 32260 Isparta, Turkey

In this study was investigated the *in vitro* antimicrobial activities against fish pathogens of the *Satureja cuneifolia* essential oil. In addition, the chemical composition of hydrodistilled essential oils of S. *cuneifolia* was analysed using gas chromatography/ mass spectrometry (GC/MS). The major constituents of *S. cuneifolia* were found to be carvacrol (46.84 %) and cymene (16.90 %). The *S. cuneifolia* essential oil was tested against *Vibrio anguillarum*, *Yersinia ruckeri*, *Aeromonas hydrophila*, *Lactococcus garvieae* isolated from rainbow trout and *Staphylococcus epidermidis* from gilthhead sea bream during disease outbreaks. Antibacterial activities of the essential oil of *S. cuneifolia* were determined by using agar well diffusion and micro well dilution assays. As a result of this study, *S. cuneifolia* oil displayed stronger inhibition zones against *V. anguillarum*, *Y. ruckeri* and *A. hydrophila* than *S. epidermidis* and *L. garvieae*. This is a critical step in determining whether such essential oils are capable of reducing mortality caused by these pathogens. However, *S. cuneifolia* oil need further study to explore its therapeutic effects against vibriosis, yersiniosis and motile aeromonas septisemi.

EFFICACY OF A POLYVALENT VACCINE AGAINST *FLAVOBACTERIUM PSYCHROPHILUM* IN ATLANTIC SALMON (*SALMO SALAR* L.)

R. HOARE[1]*, S.-J. JUNG[1, 2], T.P.H. NGO[2], K.D. THOMPSON[3], A. ADAMS[1]

[1]*Institute of Aquaculture, University of Stirling, Stirling, UK*
[2]*Chonnam National University, South Korea*
[3]*Moredun Research Institute, Pentland Science Park, Penicuik, UK*

Rainbow trout fry syndrome (RTFS) is a disease caused by the Gram-negative bacterium *Flavobacterium psychrophilum,* responsible for economic losses in the trout industry worldwide. More recently, juvenile Atlantic salmon have also been reported to be susceptible to the disease. The diversity of *F. psychrophilum* strains and the inherent difficulties in vaccinating juvenile fish has hampered the development of a vaccine for this disease. Disease episodes tend to occur between 10-14 ºC, with necrotic lesions often seen on the skin surrounding the dorsal fin and tail; in very small fish no clinical signs are apparent and death occurs due to septicaemia. At present no commercial vaccines are available, leaving antibiotics as the only course of action to contain disease outbreaks. The development of a vaccine is required, because of the potential risk of resistance developing in the antibiotics presently licenced for use in aquaculture.

The current study was performed to assess the efficacy of a polyvalent, whole cell vaccine containing formalin-inactivated *F. psychrophilum,* to induce protective immunity in salmon fry. The vaccine was formulated with a novel adjuvant containing squalene (shark oil) and aluminium hydroxide, and was compared to vaccine formulated with Montanide ISA 760VG. Duplicate groups of 21 salmon (25g) were given one of the vaccine formulations by intraperitoneal (i.p.) injection. Duplicate control groups were sham injected i.p with phosphate buffered saline. Challenge was by intramuscular injection with a homologous isolate of *F. psychrophilum* six weeks post-vaccination. Cumulative mortality reached 70% in the control fish. The fish, which received vaccine emulsified with Montanide ISA 760VG, had the lowest cumulative mortality at 3.3%, followed by fish administered vaccine alone at 10% mortality. Those fish which had received the vaccine formulated with squalene/alum reached 17.4% mortality. Initial results suggest that antibody levels prior to challenge correlate with survival post-challenge i.e. the group given the vaccine formulated with Montanide had the highest antibody levels pre-challenge and the highest survival post-challenge. However, the other groups had antibody levels the same or lower than control fish suggesting that other immune mechanisms are involved in promoting survival post-vaccination.

ALBENDAZOLE SUPRAMOLECULAR COMPLEXES AND THEIR USE AGAINST FISH HELMINTHOSES

A.V. KAZARNIKOVA*, E.N. PONOMAREVA, G.A. DUSHENKO, I.E. MIKHAILLOV

Southern scientific center of Russian academy of sciences, 41, Chekov st., Rostov-on-Don, Russia, 344006

Helminthosis, caused by the cestods *Bothriocehalus opsariichthydis, B. acheilognathi, Khawia sinensis,* occur frequently among representatives of Russian fish fauna in wild stocks and commercial rearing. Most often they are detected in carp (*Cyprinus carpio*), in silver (*Carassius auratus gibeliio)* and gold crucian carp (*C. carassius)*, as well as in grass carp (*Ctenopharyngodon idella)*, which are important species of aquaculture. The disease damages wild and cultured juveniles of these fish and the adult are parasite carriers. Despite mortality happens only due to high level of invasion, sick fish show less growth and, as a rule, - lose individual weight. Because of that the efficiency of fish farms decreases in 10-20%. Nowadays the anti-helminth drug albendazole has been testing in Russian aquaculture.

Albendazole (Albendazolum, ([5-(propylthio)-1H-benzimidazole-2yl]carbamic acid methyl ester) is a member of the benzimidazole compounds. It is characterizes by a low level of absorption, and in organism it transforms into albendazolesulfodoxid, albendazole sulfone, and albendazole 2-aminosulfone which are presented in high concentrations in plasma and tissues of fish. Albendazole has wide spectrum of activity against nematodes, cestodes and trematodes, and therefore has been successfully used in medical practice.

The disadvantage of albendazole and it known medicinal forms is their low bioavailability. To improve solubility and increase bioavailability of albendazole the soluble formulations (supramolecular complexes) are produced in order to decrease the toxicity of the active substance. By density functional theory method (DFT) B3LYP/6-311++G(d,p) theoretical investigation of structure of albendazole and relative stability of its conformational forms as well as energy barriers of their interconversions via rotation about amine and amide bonds in gas phase and in the presence of water was fulfilled. Based on the calculations the selection of appropriate partners to supramolecular inclusion complexes of albendazole were made.

The work was supported by the Russian Ministry of Education, project "Development of methods and technologies for monitoring, management and conservation of biodiversity of aquatic ecosystems in southern Russia" (№ 14.604.21.0129, unique identifier RFMEFI60414X0129).

INFLUENCE OF STERIDIAL W-15 ON GILLS STRUCTURE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

E. TERECH-MAJEWSKA[1], J. PAJDAK[1]*, E. KACZOREK[1], J. GRUDNIEWSKA[2], A. DRZEWIECKA[3], E. STRZYŻEWSKA[1], A.K. SIWICKI[2], K. DUK[1]

[1]*University of Warmia and Mazury, Olsztyn, Poland*
[2]*Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland*
[3]*Institute of Industrial Organic Chemistry Branch, Pszczyna, Poland*

Acid biocides based on organic acids and hydrogen peroxide are used in rainbow trout farms in Poland, in to protect the health of the fish.
The study was performed on fry of rainbow trout, body mass 100 g, he tested item was used in concentrations: l^{-1} of water and 16 mgl^{-1} of water. Concentrations of Steridial W-15 (s8, s16) chosen for experiment, contain in doses range of preventive treatment. Tissue samples of the gills were collected, fixed in 4% neutral buffered formaldehyde solution and embedded in paraffin. The sections were stained with hematoxylin-eosin (HE) and alcian blue-periodic acid Schiff's reagent-hematoxylin (Alcian Blue-PAS, PAB).
Dominant lesions in the gills in all exposed groups revealed by microscopic examination were hyperplasia of the respiratory epithelium, hypoplasia of mucous cells, hyperemia of the secondary lamellae and distended tips of the secondary lamellae. The infiltration of eosinophilic cells in gills was also found. However, the number of these cells was significantly higher in the groups exposed to an upper concentration of agent (s16). It appears that, although both doses have equal efficacy, lower concentrations show bigger irritant properties.
In evaluation of biocides, we take under consideration efficacy of action against pathogenic factor, and toxicity for fish and other aquatic organisms. In the light of presented results, important is the effect on tissue of gills. In practical terms it is important to use this product for shorter time and with great caution, to eliminate side effects.

INFLUENCE OF EFFECTIVE MICROORGANISMS ON OXIDATIVE STRESS BIOMARKERS IN RAINBOW TROUT

H. TKACHENKO[1], N. KURHALUK[1], E. TERECH-MAJEWSKA[2], J. GRUDNIEWSKA[3], P. SCHULZ[2], A.K. SIWICKI[3]*

[1]*Pomeranian University, Slupsk, Poland*
[2]*University of Warmia and Mazury, Olsztyn, Poland*
[3]*Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland*

Aquaculture activities are increasing worldwide. Strategies to control fish infections are urgently needed, in order to make aquaculture industry more sustainable. The effective microorganisms (EM) technology has emerged as an alternative to treat diseases and prevent the development of antibiotic resistance by pathogenic bacteria. Despite the importance and success of EM technology, little is known about the mechanisms of oxidative stress and antioxidant defense in fish during EM treating. In the present study, we determined the influence of EM on tissue-specific responses of oxidative stress biomarkers and antioxidant defense in rainbow trout (*Oncorhynchus mykiss)*. A comparative study is made concerning the oxidative stress biomarkers (lipid peroxidation and oxidatively modified protein levels), as well as activities of the antioxidant enzymes activity (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase), and total antioxidant capacity in muscle tissue, gills, liver, and heart of the rainbow trout treated by EM. In this study, feed administered to rainbow trout (150 fish per group) with initial body weight of 100 g was supplemented with 1% EM over a period of one month. Oxidative stress biomarkers analyses revealed significant differences between EM-treated and control fish. We noted strong association between oxidative stress and tissues responses. The EM-treated fish showed lower levels of lipid and protein oxidation biomarkers. EM-treated fish showed different antioxidant defense responses, likely related to tissue-specific functions. The importance of the glutathione-mediated antioxidant defense system in EM-treated trout was noted. Furthermore, changes in lipid and protein oxidation of EM-treated trout were also tissue-dependent. The results indicate that EM technology can be regarded as a new approach to control of fish condition in aquaculture systems. Considering the use of EM technology to inactivate pathogenic microbial community of aquaculture systems, the monitoring of fish condition is needed in order to select the most effective conditions.

REVEALING THE IDENTITY OF CESTODES INFECTING THE RED OCTOPUS, *OCTOPUS MAYA* VOSS & SOLIS, 1966, FROM YUCATAN, MEXICO

S. CASTELLANOS-MARTINEZ*, M.L. AGUIRRE-MACEDO

Center of Research and Advanced Studies of IPN, Campus Merida, Merida, Mexico

Octopus maya Voss and Solis, 1966 is the most important cephalopod in Mexico because of its endemic distribution and its relevance for fishery and aquaculture developed in Yucatan, Mexico. Consequently, the knowledge about the parasites harbored by this octopus species is of particular interest. Among the helminthes infecting cephalopods, cestodes are found as plerocercoid stage, which have not developed characteristics observed in adults for species identification. Larvae belonging to the order Trypanorhyncha may be identified because of their distinctive hooked tentacles. In contrast, tetraphyllidean larvae identification is more complex because their scoleces remain less differentiated until they develop as an adult inside the definitive host. Thus, the aim of the study was to identify the plerocercoid cestodes found in *O. maya* from Yucatan combining morphological and molecular approaches. Sixty-seven octopi were dissected and examined for cestode larvae. A subsample of each type of larvae was i) fixed in 10% formalin for staining with carminic acid, ii) fixed in 2.5 % formaldehyde for Scanning Electron Microscopy (SEM) and, iii) preserved in 96% ethanol for further amplification of nuclear 28S lsrDNA. According to morphology analyzed by light microscopy, four plerocercoid morphos were identified: *Prochristianella hispida*, *Phyllobothrium* spp., *Echeneibothrium* spp., *Othobothrium* spp. and *Nybelinia* spp. The identity of the plerocercoids at generic level was confirmed by SEM, except for *Phyllobothrium* spp. So far, the identity of *P. hispida* has been confirmed through analysis of hook tentacles and amplification of 28S 1srDNA. With molecular analysis still ongoing it is expected to reach the specific identity of the rest of plerocercoids. The molecular identification will increase the available sequences in public databases of cephalopod parasites that may in turn contribute to future phylogenetic studies of cestodes infecting cephalopods.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

MORPHOLOGICAL CHARACTERIZATION OF DICYEMID MESOZOANS (PHYLUM DICYEMIDA) OF *OCTOPUS MAYA* VOSS AND SOLIS 1966 FROM YUCATAN, MEXICO

S. CASTELLANOS-MARTINEZ*, L. AGUIRRE-MACEDO

Center of Research and Advanced Studies of IPN, Campus Merida, Merida, Mexico

Dicyemid mesozoans are common endosymbionts inhabiting the renal appendages of cephalopods, they are characterized by a simple morphology with low number of cells and a complex life cycle. Dicyemids has been suggested as natural tags to assess cephalopod stocks and currently they are used to assess host population structure. The present study describes for the first time dicyemid species from *O. maya*, an endemic octopus from the Yucatan Peninsula, Mexico. Octopus samples were collected from the artisanal fishery at four localities: Celestun (n=6), Sisal (n=33), Progreso (n=15) and Ria Lagartos (n=8). Hosts renal sacs were dissected and fifteen smears were made from each (right and left) sac, fixed in Bouin fluid and stained with hematoxilin-eosin. There were no infection differences between right and left renal sacs. The calotte of all found dicyemids was conical in shape, formed by 4 propolar and 4 metapolar opposite cells, which is a taxonomic character of the genera *Dicyema*. The body length of dicyemids from Celestun, Sisal and Progreso did not exceed 700 µm while those from Ria Lagartos reached up to 1685 µm. Dicyemids from Celestun shows a strong body constituted by 20 peripheral cells, and the axial cell extended up to the middle of metapolar cells. The axial cell of dicyemids from Sisal, Progreso and Ria Lagartos extends up to the base of the calotte. However dicyemids from Sisal and Progreso had only 16 peripheral cells while those from Ria Lagartos observed 22-24. Vermiform embryos from Celestun, Sisal and Progreso were similar in total length (60 µm) in contrast with Ria Lagartos (83 µm). Nevertheless, differences were found in the number (4, 2, 2, 2 respectively) and position of axoblasts. According to our results, different dicyemid species inhabit the renal sacs of *O. maya* along its distribution. A dicyemid species is shared by the closest localities (Sisal and Progreso) whereas a different one is harbored by *O. maya* at Ria Lagartos. With respect to specimens found at Celestun additional samples needs to be examined in order to find additional characters that allows dicyemids differentiation from this locality with respect to the other ones.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

DIFERENTIAL EXPRESSION OF *OCTOPUS VULGARIS* IMMUNE GENES IN RESPONSE TO THE GASTROINTESTINAL COCCIDIA, *AGGREGATA OCTOPIANA* INFECTION

S. CASTELLANOS-MARTÍNEZ, C. GESTAL*

Institute of Marine Research (IIM-CSIC), Vigo, Spain

The gastrointestinal coccidia *Aggregata octopiana* is considered the most dangerous parasite for wild and reared octopuses. The negative effect of coccidiosis on the octopus immune response has been proved at biochemical and functional level. Moreover, molecular evidence is being provided in order to understand how this host-parasite interaction occurs. The aim of the current work is to analyze the differential expression of immune genes in octopuses harboring different level of coccidiosis. Two octopus groups were formed according to tissue damage assessed by histology and the number of *A. octopiana* sporocyts per gram of digestive tract tissue: sick and healthy. The appropriate reference gene was selected after assessing the stability of the genes: [glyceraldehyde 3-phosphate-dehydrogenase (GADPH), β-actin (ACT1), β-actin (ACT2), ubiquitin (UBI), elongation factor 1 alpha (EF1-α), elongation factor 1 alpha (EF2-α), β-tubulin (TUB)]. The expression stability was analyzed using the software NormFinder, GeNorm and Bestkeeper. The expression analysis was performed using RNA extracted from hemocytes and caecum, and quantified by RT-qPCR. After the sequencing of a *de novo* transcriptome of octopus hemocytes, six genes involved in pathogen recognition (TLR, C1q and PGRP), oxidative stress (PRDX), apoptosis (Caspase 3) and a serine protease inhibitor (SERPIN) were selected to test the differential expression in sick and healthy octopuses according to Pfaffl method. Most of the genes were down-regulated in hemocytes from sick octopuses except TLR suggesting that parasitic recognition is active, but additional defensive mechanisms might be weaken. Contrarily, the genes tested were up-regulated in caecum, suggesting than recognition of pathogens and cytotoxic mechanisms might be actively working to face infections.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

IMMUNE RESPONSE OF COMMON OCTOPUS *OCTOPUS VULGARIS* FOLLOWING BACTERIAL INFECTION

S. CASTELLANOS-MARTÍNEZ, C. GESTAL*

Institute of Marine Research (IIM-CSIC), Vigo, Spain

Wild and reared cephalopods are affected by a wide variety of pathogens, mainly bacteria, protozoan and metazoan parasites. One of the most recent records of bacterial infections in wild cephalopods was the Gram-negative bacteria *Vibrio lentus*. This bacterium was reported in *Octopus vulgaris* kept in floating cages at the Ría of Vigo causing mortalities. The effect of live bacteria *V. lentus* on the phagocytic and respiratory burst (ROS) activity of the octopus hemocytes, and the expression of specific immune related genes was studied. Respiratory burst and phagocytic ability of hemocytes were measured by flow cytometry after 1, 4 and 24 h of injection with live bacteria. A general *de novo* transcriptome sequencing of octopus hemocytes previously generated using high-throughput Illumina technology allowed the identification of a repertory of genes involved in immune functions. A total of 6 immune related genes involved in pathogen recognition (TLR, C1q and PGRP), oxidative stress (PRDX), apoptosis (Caspase 3) and a serine protease inhibitor (SERPIN) were selected to study the molecular immune response of *Octopus vulgaris* hemocytes following infection by *V. lentus*. RT-QPCR was performed and the expression was determined. The phagocytic ability of the octopus hemocytes was affected by the infection, decreasing during the first hour of infection, when the lowest engulfment of fluorescent beads was recorded, compared to non-infected octopus, whereas ROS production shows the lowest level after 4 h p.i. Concerning molecular analysis, the results showed that most of the tested genes were up-regulated after infection, and the expression level was higher at 1 and 4 h post-infection, showing a decrease after 24 h p.i., except SERPIN, that showed the highest expression level at 4h p.i. The present results suggest the ability of common octopus immune system to recognize and fight the pathogenic bacterial infection.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

PHYLOGENY AND Q-PCR DETECTION OF *AGGREGATA OCTOPIANA* (AGGREGATIDAE, APICOMPLEXA) PARASITISING DIGESTIVE TISSUES OF COMMON OCTOPUS (*OCTOPUS VULGARIS*) FROM THE ADRIATIC SEA

K. BEGIĆ[1], M. PETRIĆ[2], I. MLADINEO[1]*

[1]*Institute of Oceanography & Fisheries, Split, Croatia*
[2]*University Department of Marine Studies, Split, Croatia*

Aggregata octopiana (Aggregatidae, Apicomplexa) is the most prevalent coccidian in the wild common octopus (*Octopus vulgaris*), whose heteroxenous life cycle includes gamogony and sporogony in the octopus digestive tract. In the intensively infected reared octopi, an unusual extraintestinal distribution of the coccidian, with both gamogony and sporogony ongoing in dermal and gill tissues can be observed, often leading to mortalities in the aquaculture environment. Although with a 100% prevalence in adult octopi in the wild, there is scarce data concerning phylogenetic diversity of *Aggregata* sp. in the Adriatic Sea. Recently, a study analysed a locus of 18S rRNA gene of *A. octopiana* from the NE Atlantic coast and compared it to a single sequence previously isolated from the Adriatic Sea, revealing considerable genetic distance between two *A. octopiana* lineages that suggested the existence of two species. Therefore, the aim of our study was to isolate apicomplexan from wild octopi sampled through wide geographical area of the East Adriatic Sea and asses its phylogenetic relationship within and outside the Adriatic using PCR. For the analysis, we used two primer pairs that amplified 1000 and 600 bps of partial 18S rRNA sequence, respectively. Faced with an often varying success of locus amplification by PCR, being correlated with the scarce quantity of apicomplexan oocysts, we also developed a q-PCR assay for more sensitive quantification of the parasites in octopus tissues. Phylogenetic analysis of *A. octopiana* population evidenced a panmictic character of the parasite within the Adriatic, while comparison with Atlantic lineages supported previous hypothesis of potential species differentiation. Q-PCR proved to be a successful tool for quantification of low-level infections.

This presentation is considered as contribution to the activities of the COST Action FA1301 - CephsInAction.

POTENTIAL LIMITING FACTORS TO THE GROWTH OF *OCTOPUS VULGARIS*

P. TEDESCO[1, 2]*, E. BALDASCINO[2], G. DE MARTINO[2], G. FIORITO[2]

[1]*Università del Salento, Lecce, Italy*
[2]*Stazione Zoologica Anton Dohrn, Naples, Italy*

Octopus vulgaris is a highly valuable food resource and good candidate for marine aquaculture. Over the past years, more than a hundred of published papers have been devoted to increase the growth performance of captive octopuses and overcome the problems associated with the adaptation of these animals to farming conditions. Despite this, the farming of *O. vulgaris* is still far from being a large-scale, economically profitable activity due to high costs, heavy losses in terms of mortality and inadequate growth.
In order to investigate possible factors limiting growth of animals, we will present our historical long-term and experimental data on growth of *O. vulgaris* in standardized uniform maintainance conditions and correlate growth of the animals with season/temperature, genetic factors, possible occurrence of a malabsorption syndrome due to parasitic infections. The study is based on the retrospective application of the Standardized Fulton Condition Index on historical and experimental data as a good non-invasive predictor of bias in the animals'growth.

This presentation is considered as contribution to the activities of the COST Action FA1301 – CephsInAction.

MOLICOLA HORRIDUS (GOODSIR, 1841) DOLLFUS, 1935 (CESTODA, TRYPANORHYNCHA) ENDOPARASITIC PATHOGEN OF THE OCEAN SUNFISH, *MOLA MOLA* (L.)

A.E. AHUIR-BARAJA*, A. REPULLÉS-ALBELDA, J.A. RAGA, F.E. MONTERO

Institute Cavanilles of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Valencia, Spain

Molicola horridus (Goodsir, 1841) Dollfus, 1935 is a trypanorhynch cestode whose *plerocercus* larvae are present in liver, muscle and kidney of the ocean sunfish, *Mola mola* (L.). To study the pathology of this parasite 106 fish from La Azohía (Cartagena, Murcia, Spain) and Canet de Berenguer and Almazora (Valencian Community, Spain) were analysed. The fish were divided into two groups: 101 "small" fish (less than 1m) and 5 "large" fish (more than 1m). The liver, muscle and kidney were analysed, calculating the intensity and prevalence. Biomass, density and percentage of parasitized hepatic surface were also calculated, as these worms are extremely long and traditional ecological parameters could underestimate the parasite load. The histopathological analysis of the target organs is also given.

From the three analysed organs, the liver was the most parasitized location, with the highest intensity, prevalence, biomass and density. The hepatic parasitized surface reached up to 28.3% (9.4±6.6).The histological studies of the affected organs did not show a significant inflammatory response related to the presence of the parasite. Only in kidney the inflammatory response was more patent than in liver and muscle.

The usual high infection levels and the apparent healthy aspect of the infected fish seem to indicate that the effect of the parasite is mostly harmless. However, the high parasite loads in the liver must be analysed carefully to detect sublethal effects, as parasite presence could provoke partial hepatic dysfunction and physiological stress.

Funded by AGL2010-20892, ISIC/2012/003 & PROMETEO 795/2015/018 projects.

FIRST OUTBREAK OF CYPRINID HERPESVIRUS 2 (CYHV2) ON GOLDFISH (*CARASSIUS AURATUS*) IN FRANCE

P.-M. BOITARD[1], M. BAUD[2], S. LABRUT[3], C. DE BOISSÉSON[4], M. JAMIN[1], L. BIGARRÉ[2]*

[1]*Fili@vet – aquacole veterinarians, Saint-Martin-des-Champs, France*
[2]*ANSES, Plouzané, France*
[3]*Oniris, Nantes, France*
[4]*ANSES, Ploufragan, France*

Massive mortalities of *Carassius auratus* (L.) occurred in a farm in France during summer 2014. Fish presented anorexia, loss of scales and large amounts of mucus on the gills. Necrosis of the distal tip of the filament and the lamellae, combined to the fusion of the lamellae, were observed, as well as necrosis in the hematopoietic organs and in the digestive tract. First complementary exams lead to hypothesize the implication of a virus in the mortality, the bacteria being rather opportunistic agents. The presence of Cyprinid herpesvirus 2 (CyHV2) in dead fishes was demonstrated by the amplification and sequencing of portions of the DNA polymerase and helicase genes, both sequences exhibiting 100 % identity with CyHV2 from Japan. In an attempt to find genetic markers of variation, two regions containing tandem repeats in the Japanese genome were amplified from a virus-positive sample from the present outbreak. A first region (mB) was fully identical to the Japanese isolate. However, the second region (mA) exhibited a range of deletions and substitutions compared to CyHV2 from Japan. This is the first report of CyHV2 in France, in association with mortality of goldfish, and the first identification of a molecular marker for its tracing.

MYCOBACTERIA SPECIES IDENTIFIED IN AQUARIUM FISH FROM SWEDISH WHOLESALERS

T. HONGSLO*, E. JANSSON

National Veterinary Institute, Uppsala, Sweden

A health survey of aquarium fish from Swedish pet-shops, carried out 2006 to 2007, showed that one of the most common causes of diseases was infection with acid-fast bacteria (Hongslo & Jansson, 2009). Characterization of these bacteria to the species level by molecular genetic technique revealed that *Mycobacterium (M.) marinum* was the most common species in the fish from the pet-shops (Hongslo & Jansson, 2014). The aim of this study was to examine the occurrence of different species of mycobacteria in aquarium fish from wholesalers in Sweden. Three groups of commonly occurring aquarium fish species: 1) Guppy (*Poecilia reticulata*), 2) Neontetra (*Paracheirodon innesi*) and 3) Fantail goldfish (*Carassius auratus auratus*) or Ramirez's dwarf cichlid (*Microgeophagus ramirezi*) from eight wholesalers were investigated. Thirty fish of each species from each wholesaler were randomly collected, followed by cultivation of pooled tissue materials from each of ten fish for two months. DNA was extracted from growing bacterial colonies and amplified by PCR. The 16S rRNA and *rpo B* genes were Sanger sequenced and the consensus sequences were matched against known sequences of different bacteria species, using the database GenBank (http://www.ncbi.nlm.nih.gov). The results showed mycobacteria in fish from in total six (75%) of the eight wholesalers. Mycobacteria species were detected in four (50%) of the eight Guppy groups, in four (50%) of the eight Neontetra groups and in two (25%) of the eight Fantail goldfish/ Ramirez's dwarf cichlid groups. The most common mycobacteria species identified was *M. marinum*, which was detected in fish from three wholesalers (solely in three Guppy groups), followed by *M. chelonae* and *M. gordonae*, which both were found in fish from two wholesalers (in all three fish groups). In summary, this study showed that *M. marinum* was the most frequent mycobacteria species in commonly occurring aquarium fish from Swedish wholesalers. This finding is in agreement with our previous study, showing that *M. marinum* also was the most common mycobacteria species in diseased aquarium fish in Swedish pet-shops. It is therefore obvious that *M. marinum* is of significance importance in aquarium fish as a fish pathogenic agents, a bacterium also known to cause fish tank granulomas in humans.

2014 DISEASE SURVEILLANCE OF WILD MARINE FISH CAUGHT FROM INSHORE-OFF SHORE OF KOREA

M.A. PARK[1]*, K.I. KIM[1], M.Y. CHO[1], B.Y. JEE[1], J.H. CHOI[2], J.C. KO[2], S.D. HWANG[1]

[1]*Aquatic Life Disease Control Division, National Fisheries Research and Development Institute, Busan, South Korea*
[2]*Fisheries Resources Management Division, National Fisheries Research and Development Institute, Busan, South Korea*

Disease surveillance in wild fish is necessary to obtain information about disease occurrence, disease agents, and the transmission of diseases between wild and cultured species. In this context, we performed a monitoring of viral and bacterial diseases in wild marine fish and crustacean caught by trawl in southern coastal waters of Korea in March and November, 2014. We monitored viral diseases in 660 individual fish from 41 different species and 157 individual crustaceans from 12 different species. The organs we monitored were kidney or spleen to detect viral haemorrhagic septicemia virus (VHSV), red sea bream iridovirus (RSIV), viral nervous necrosis (VNN) and marine birnavirus (MBV). We also monitored hepatopancreas of crustaceans to detect infectious hypodermal and hematopoietic necrosis (IHHN), infectious myonecrosis (IMN), taura syndrome virus (TSV) and white spot disease (WSD). As a result, only a few virus diseases were detected in the tested samples: VNN in three yellow goosefish (*Lophiius litulon*) and TS in one lady crab (*Ovalipes punctatus*). Phylogenetic analysis was conducted based on the nucleotide sequences of virus isolated from various species in order to investigate their genetic relationship. Surveillance of bacteria diseases was also made using collected fish. A total of 79 of bacteria were isolated from kidney or spleen and dominant species among them were *Pseudoaltermonas* sp. (16.5%), *Vibrio* sp. (13.9%), and *Photobacterium* sp. (12.7%). The rate of bacteria isolation in March (20.6%) was higher than that in November (7.6%). These results will help control the diseases and identify disease-free zones in Korea.

ATRIASTER LEBEDEV ET PARICHIN, 1969 (MONOGENEA: MICROCOTYLIDAE) FROM OMANI WATERS: AN OUTLOOK INTO SPECIES COMPOSITION AND DISTRIBUTION ALONG COAST

V. MACHKEVSKYI[1], S. AL-JUFAILI[1, 2], U. AL-KINDI[1]*, R. KHALFAN[1], N. AL-MAZROOEI[1]

[1]*Fishery Quality Control Centre, Ministry of Agriculture and Fisheries Wealth, Muscat, Sultanate of Oman*
[2]*Rostock University, Rostock, Germany*

The new genus *Atriaster* Lebedev et Paruchin, 1969 was erected to accommodate a new species of microcotylid monogeneans *Atriaster heterodus* Lebedev et Paruchin, 1969, found on the gills of Sparid fish *Sparus heterodus* (Sparidae) near Walvis Bay (South West Africa). The second species of this genus *Atriaster salpa* (Parona et Perugia, 1890) Ktari, 1969 was originally described as *Microcotyle salpa* from *Sarpa salpa* (Sparidae) from the Mediterranean Sea. Later (1975) in the Arabian Sea off the coast of Oman and Yemen three species of this genus *A. spinifer*, *A. acanthopagri*, *A. bifidacanthus* were described from Sparidae fishes (*Argyrops spinifer*, *Acanthopargus bifasciatus*, *Sparus* sp.) (Mamaev et Paruchin, 1975). Additionally, *Microcotyle sargi* Parona et Perugia, 1889 (Mamaev et Paruchin, 1975) parasitizing *Sargus sargus* (Sparidae) in the Mediterranean Sea was assigned to the same genus. Almost simultaneously, this group joined another species *seminalis* from the Atlantic Ocean, as a parasite of sparids in French waters (Euzet, Maillard, 1974). From the obtained information, we can conclude that at present the genus *Atriaster* has only 6 species exclusively parasitizing fish belonging to the family Sparidae. The number of published works on this species of monogeneans is less than ten, which gives us enough reason to assume that this is an under studied group of monogenean parasite and lead us to further investigate it from Omani waters. As far as we know, comprehensive parasitological investigations are almost non existing in Oman (Paruchin, 1976, 1989) and since 16 sparid species are known inhabit Omani waters (Randal, 1995) we present the results of the species composition and distribution of *Atriaster* along the coast of Oman from its Southwest (Salalah) to the Northeast (Musandam) coasts. The parasitological parameters of the invasion and host specificity of the genus *Atriaster* monogeneans are presented.

SEASONAL DYNAMICS OF ERGASILOSIS IN FISH IN SELECTED WATER RESERVOIR DEPENDING ON ZOOPLANKTON DEVELOPMENT

S. NAVRÁTIL*, E. JELÍNKOVÁ, Z. MARKOVÁ, I. PAPEŽÍKOVÁ, M. PALÍKOVÁ

University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

The objective of the work was to evaluate seasonal dynamics of ergasilosis in fish in water reservoirs Hubenov and Koryčany depending on zooplankton development. This study is based on the results of fish examination and monitoring of zooplankton in selected water reservoirs in the period from April to October 2014. Total amount of 189 fish of 10 species during 7 catches in each of the water reservoirs were examined. Additionally, 7 samples of pelagic zooplankton were collected from each water body monthly. The catches were conducted with an electric aggregate and gill net. The results were evaluated using epidemiological characteristics such as infection intensity, prevalence and abundance.

In the water reservoir Hubenov the highest infection intensities and abundance were recorded in June and September. The growth of values in June probably suggests an attack of the 1st generation of the parasite and in September the 2nd generation of the parasite. In the water reservoir Koryčany the highest values were recorded as early as in May which may be probably related to the higher water temperature and faster parasite development. In September and October the values increased again suggesting presence of the 2nd generation of the parasite. In both water reservoirs the values decreased in July and August. The prevalence was in the range 33 - 100 %. The highest intensities of infection were found in asp (*Aspius aspius*) and northern pike (*Esox lucius*).

Every water reservoir is a unique ecosystem and its seasonal development of ergasilosis is specific. Differences are probably associated with the growth of zooplankton which is affected by fish stocking in the given water reservoirs. As zooplankton includes nauplius and copepodite stages of parasite the level of infestation should be higher in the water reservoirs with a lower predatory pressure on zooplankton, i.e. in those bodies with more carnivorous fish that suppress zooplanktonophagous fish. In this respect, fish stock is functional to maintain better quality of water; nevertheless even in this case ergasilosis can develop rapidly.

HISTOLOGICAL CLASSIFICATION OF GONADIC NEOPLASMS IN KOI CARP (*CYPRINUS CARPIO*) FROM BELGIUM AND ITALY

R. SIRRI[1]*, L. MANDRIOLI[1], T. BARBÉ[2]

[1]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy*
[2]*Veterinary practitioner, Lennik (Vlaams-Brabant), Belgium*

Samples of coelomatic masses from 8 koi carps (*Cyprinus carpio*), 5 females and 3 males, were histologically investigated. The animals were referred to the veterinary practitioner for an evident abdominal enlargement. Macroscopically, the coelomatic masses appeared multilobulated, white-yellowish with multifocal hemorrhages, sometimes soft and cystic with conspicuous amount of yellow transparent fluid, or firm and solid. Frequently they involved the other organs (liver and intestine) and sometimes residual gonadal tissues was recognizable.
Histologically they displayed different patterns: 5 neoplasms were composed of admixed cell neoplastic germ and sex-cord cells arranged in lobules and bundles with thick fibro-collagenous septa. The germ cells were round to polygonal cells, with indistinct cell borders, abundant clear to eosinophilic granular cytoplasm, and round central nucleus and non-visible nucleoli. The sex cord component was composed of elongated cells with indistinct cell borders, scarce eosinophilic cytoplasm, often vacuolated, oval to fusiform nucleus and multiple magenta nucleoli. Mitotic rate was 4-5 per HPF. The diagnosis was mixed germ cell sex cord-stromal tumor. One neoplasm was composed of bundles of fusiform cells sharing similar characteristic of the sex cord component above described. In this case the diagnosis was sex cord-stromal tumor. Another neoplasm was composed of trabeculae and cords of polygonal cells sustained by abundant fibrous septa. The cells had distinct cell borders, scant to moderate amount of eosinophilic cytoplasm, round central nucleus and single or multiple nucleoli. Mitotic rate was 3 per HPF. The diagnosis was ovarian carcinoma. The last neoplasm was composed by sheets of polygonal cells, supported by fine fibrovascular stroma, with distinct cell borders, scant granular eosinophilic cytoplasm, large central round hypochromatic nucleus and single nucleolus. The mitotic rate was <1 HPF. The diagnosis was seminoma. In all the neoplasms multifocal and large areas of necrosis were frequently present. Residual spermatids or previtellogenic oocytes were admixed to the neoplastic cells.
Literature about classification of gonadal neoplasms in koi carps is scant and diagnosis is still challenging and based mainly on the veterinary WHO classification. Recently an epidemiological survey on internal neoplasm of koi carp in Switzerland has been reported (Ott Knüsel et al., in press).

CHARACTERIZATION OF VIRULENCE GENES AND PATHOGENICICTY OF MOTILE AEROMONADS ISOLATED FROM KOI CARP (*CYPRINUS CARPIO KOI*) AND THEIR WATER ENVIRONMENT

M.A. TSAI[1]*, P.C. WANG[1], C.W. CHO[1], Y.S. HO[2], S.C. CHEN[1]

[1]*College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*
[2]*Eastern Marine Biology Research Center, Fisheries Research Institute, Council of Agriculture, Taitung, Taiwan*

Motile aeromonads such as *Aeromonas hydrophila*, *A. sobria* and *A. caviae* are among abundant bacteria found in fresh water aquatic environments and capable of producing disease in fish. Total 45 isolates of *Aeromonas* spp. were collected from diseased koi carp (*Cyprinus carpio koi*) with hemorrhages and ulcerations on the body surface and their water environment in Taiwan during 2013 to 2014. All of them have been identified as *A. hydrophila* (n=13 from fish, n=5 from water), *A. caviae* (n=12 from fish, n=5 from water) and *A. sobria* (n=10 from fish) by PCR. The presence of five virulence genes include aerolysin (aer), polar flagella (flaA and flaG), nuclease (nuc) and lateral flagella (laf) of *Aeromonas* spp. isolates were detected by PCR method. It was observed that aer gene was the most common gene among all *Aeromonas* spp. isolates from fish (90-92.3%) and water (80%). The genes encoding polar flagella, flaA and flaG, were 20% to 41.7% in fish isolates, whereas 60-80% in water isolates. In pathogenicity study, koi carp were injected intramuscularly with 3×10^7 cfu $fish^{-1}$ of 8 *Aeromonas* spp. strains from koi carp and water sample, respectively. Three water strains (1 *A. hydrophia* strain and 2 *A. caviae* strains) and one *A. caviae* strain from fish displayed low mortality (0-20%) in koi carp. Conversely, *A. sobria* and *A. hydrophila* strains from fish displayed a mortality rate of 70-100% and caused skin lesions similar to those observed in naturally infected koi carp. These results showed that the *A. sobria* and *A. hydrophila* are pathogenic to koi carp, and causing koi ulcer cases in Taiwan.

ARRAINA: WORKSHOP ON NUTRITION AND IMPLICATIONS ON AQUATIC ANIMAL HEALTH

Presentations: from O-031 to O-036

D. MONTERO

Grupo de Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain

Nutrient balance in diets and feeding regimes play critical roles in intensive aquaculture and the development of nutritional strategies that positively influence immunity and disease resistance of cultured organisms has special importance to reduce disease-related economic losses. The availability of specific nutrients in terms of deficiencies can compromise the immunocompetence of culture fish, as well as the unbalanced ratios among different nutrients, whereas dietary supplementation of some nutrients has been shown to significantly enhance immune responses and disease resistance. It is of special importance to understand the mechanisms involved in the roles of dietary nutrients in modulation of pathogenesis and infection in fish. This is of special importance under changes of dietary regimes such as long-term feeding with alternative feeds replacing fish meal and fish oil in diets for fish.

INTRODUCTION TO MENDELEY, THE MOST POWERFUL REFERENCE MANAGER

B. GORGOGLIONE[1, 2]

[1]*Clinical Division of Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, Vienna, Austria*
[2]*Mendeley Advisor*

Mendeley free reference manager is the most powerful addition to your research workflow! References can now be easily imported with just one click using the Web-Importer tool or by moving PDFs into the folders you want Mendeley Desktop to watch on your computer. Mendeley will automatically organize your paper collection and syncing your account between multiple computers. Using the MS Word Plugin all your references become quickly available to be cited in a text, providing a great help when writing a scientific paper or report. In a few seconds the citation style and the bibliography are automatically arranged according to the journal style chosen. Workshop attendees will discover and practice references quick importing, library organization, how searching text across papers and about documents sharing with colleagues. Upon free registration Mendeley provides 2 GB of free web storage space to keep documents always accessible online. This workshop, sponsored by Mendeley, is specially designed to introduce the potential of this free software to new users and to provide a better knowledge of potential resources to people already using it. Particularly targeted to senior scientists and students it will provide basic teaching on how facilitate and improve your everyday writing activity. During the workshop will be launched the official citation style adopted for publishing on the Bulletin of the European Association of Fish Pathologists.

NOVEL VIRAL INFECTIONS IN CYPRINID FISH

O. HAENEN[1]*, K. WAY[2]*, T. ITO[3]*, T. WALTZEK[4]*

[1]*Leading organizer, Central Veterinary Institute of Wageningen UR, NRL for Fish, Crustacean and Shellfish Diseases, Lelystad, The Netherlands*
[2]*Co-organizer, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth DT4 8UB, England*
[3]*Tamaki Laboratory, Aquatic Animal Health Division, National Research Institute of Aquaculture (NRIA), Fisheries Research Agency, Mie 519-0423 Japan*
[4]*Co-organizer, College of Veterinary Medicine, Department of Infectious Diseases and Pathology, University of Florida, Gainesville, FL 32611-0880, USA*

In this open workshop (approximately 1.45h) novel viral infections of cyprinid fishes will be discussed including Carp Edema Virus (CEV), *Cyprinid herpesvirus 2* (CyHV-2), and others.
Each participant receives a handout. After an introduction by O. Haenen, an overview of several new viruses found in cyprinids will be presented by T. Waltzek. Then, Carp Edema Virus will be discussed by K. Way. CEV has been detected in several countries in *Cyprinus carpio* (carp and koi) in Europe since 2004 - an overview and its possible impact will presented. Subsequently T. Ito from NRIA, Japan will present a lecture on goldfish herpesvirus known formally as *Cyprinid herpesvirus 2* (CyHV-2).
Program:

- Introduction (Olga Haenen)
- Various new cyprinid viruses (Thomas Waltzek)
- Carp Edema Virus (CEV) (Keith Way)
- CyHV-2 (Takafumi Ito)

Then, there will be 4 other lectures of each 6 min, and a discussion.
One expected outcome from the workshop is the writing of a joint publication for the EAFP Bulletin about the topics covered on novel viral infections of cyprinid fishes.
We like to invite you to participate in this workshop.
Discusion points, among others:

- Which are the most important novel viral disease problems in cyprinid fishes, apart from KHVD and SVCV?
- Are diagnostic methods up to date, which ones to use?
- Are prevention measures in place, and which specific vaccines are needed?
- Which recommendations can be made for an adequate prevention of novel viral diseases in cyprinid fishes?

EUROPEAN DIRECTORATE FOR THE OF THE MEDICINES AND HEALTH CARE (EDQM): EUROPEAN PHARMACOPOEIA REQUIREMENTS FOR FISH VACCINES

Presentations: O-096, O-097, P-133 and P-134

Ø. EVENSEN, C. LORTEAU

[1]*Norwegian University of Life Sciences (Norway)*
[2]*French Agency for Food, Environmental and Occupational Health & Safety, France*

FISH HEALTH IN MEDITERRANEAN AQUACULTURE, PAST MISTAKES AND FUTURE CHALLENGES

N. VENDRAMIN[1], D. ORAIĆ[2], S. ZRNČIĆ[2], F. PADROS[3]

[1]*DTU Veterinary Institute, Copenhagen, Denmark*
[2]*Croatian Veterinary Institute, Zagreb, Croatia*
[3]*UAB-University of Barcelona, Spain*

A significant production in European aquaculture consists of European sea bass and gilthead sea bream. According to EAS data, approx. 135.000 tons of sea bass and 139.000 tons of sea bream were produced in 2012 in the Mediterranean basin. Despite the relevance of these species, both in terms of production and economic value, no specific provision is given in the legislation regarding the survey of important diseases. In this framework the information available about infectious diseases and priorities to further develop this production are quite fragmented and jeopardized. Since 2012 the EURL started a survey involving a number of private and institutional experts depicting the most important diseases in the Mediterranean. Each year the data are compiled and presented at the EURL Annual Workshop, but the increasing relevance of the health aspects in the Mediterranean aquaculture seems to require a more accurate and comprehensive interpretation exercise with as much professionals as possible involved in it. The initiative of this workshop was build in order to create a discussion forum using a specific working methodology.
This workshop aims to integrate the efforts and the knowledge already generated with other undergoing activities carried out by other organization (i.e. EATip, EAS and FEAP) with the knowledge of experts and workshop participants. On beforehand and after a short introduction, participants will be provided with an open list of known and emerging pathogens. Participants will have to discuss and rank the 3 most important diseases for the relevant fish Mediterranean species a detailing the reason/background behind this choice. The criteria that should guide the ranking of the different diseases should focus and rely on the reduction of income that a single disease, or a complex of diseases, determines on the expected production programme. The final output of the Workshop is an agreed working document to be used as a baseline for future initiatives, ranking and prioritizing the most important diseases in the Mediterranean area according to the economic impact.

PUBLISH OR PERISH – EVERYTHING YOU WANTED TO KNOW ABOUT SCIENTIFIC PUBLISHING BUT WERE AFRAID TO ASK – SCIENTIFIC PUBLISHING WORKSHOP

B. NOWAK[1], S. POYNTON[2]

[1]*University of Tasmania, Launceston, Australia*
[2]*Johns Hopkins University, Baltimore, USA*

Communicating results of scientific research through publications is an essential part of academic life. Quality and quantity of published papers is one of the way academics are evaluated for jobs, promotions and research funding. Most people start their academic career by publishing scientific papers in international journals, yet have little prior experience of scientific writing, limited knowledge of the publication process, and may not have much support. While most journals now provide important information on their websites there are still some aspects of publishing which are important from the point of view of the author, but not fully explained. This workshop will focus on what happens after a paper is submitted, potential outcomes, and how to deal with rejection. Retractions and errata will also be covered. Ideas for how to improve publication rate will be discussed. The workshop will consider the responsibility and authority of the reviewers, associate editor and editor. The workshop is for MSc and PhD students, and early career researchers, who want to learn more about how to be successful with publishing papers. The delivery is through presentation and discussion. The workshop leader, Dr. Barbara Nowak, is Associate Editor of Journal of Fish Diseases and a member of editorial boards for a number of other journals. Dr. Sarah Poynton is a reviewer for a diversity of aquatic animal health journals, teaches scientific and biomedical writing, and is a freelance editor.

AMOEBIC GILL DISEASE WORKSHOP

B. NOWAK[1], H. RODGER[2]

[1]*University of Tasmania, Launceston, Australia*
[2]*Vet-Aqua International, Oranmore, Ireland*

Amoebic gill disease (AGD) is a condition affecting some species of farm-reared marine fish caused by *Neoparamoeba perurans*. AGD was initially reported only in Australia and USA but by now it has a significant impact on salmon production in Australia, Scotland, Norway and Ireland. Main treatments used commercially include fresh water and hydrogen peroxide. A range of other treatments are in experimental trials. Other management strategies such as use of vaccines and immunostimulants are being explored. AGD has been observed in a range of fish species farmed or held in the marine environment including wrasse and lumpsucker, which are used as cleaner fish to control sealice on Atlantic salmon. There is limited knowledge about the causative agent *Neoparamoeba perurans*. Reservoir populations of the amoeba and the mechanism of transmission to farmed fish have not been elucidated. While it is present in water it is only at a very low concentration, even on the affected salmon farms. Preliminary investigation showed negative results for sediments and biofouling organisms in AGD affected area in the USA. However *N. perurans* DNA was detected in alcoholic washings of salmon lice *Lepeophtheirus salmonis* collected from salmon from an affected farm in the USA. It has also been detected in biofouling organisms and *L. salmonis* in Ireland. Furthermore cross-infection with another species of sea lice *Caligus rogercresseyi* was reported during an AGD outbreak in Chile, however it was not clear if there was any synergistic effect and if the mortalities were due to AGD or the sea lice infection. This suggests that epidemiology of this disease may depend on the geographical locations. The effect of the disease on host at gene and protein level as well as AGD pathology will be discussed. This workshop will review and summarise our current knowledge of this disease and discuss research priorities.

MOLECULAR TRACING OF VIRAL DISEASES IN AQUACULTURE

N.J. OLESEN[1], B. BANG JENSEN[2], J.-C. AVARRE[3]

[1]*EURL for Fish Diseases, DTU Vet. Denmark*
[2]*NVI, Norway I*
[3]*IRD, France*

This workshop will focus on new findings and tools for molecular tracing of viral diseases in aquaculture. The primary aim of the workshop is to bring colleagues involved in molecular biology, bioinformatics and epidemiology together in order to discuss improved methods for risk analysis and prevention of spread of serious diseases in aquaculture.
The workshop will last 1.45 hours.
Preliminary program:

- Molecular tracing of viral pathogens in aquaculture - a review
- B. B. Jensen, NVI: Scenario simulation models for control options
- B. Kristoffersen, NVI: Use of sequence data in epidemiological analysis
- V. Panzerin; IZSVe, Evolution for VHSV and IHNV in Italy
- N.J. Olesen, DTU facilitator: Round table discussions

INDUSTRY ROUNDTABLE: TARGETFISH

P. SMITH[1], G. WIEGERTJES[2]

[1]*Tethys Aquaculture Ltd, Essex, UK*
[2]*Wageningen University, Wageningen, The Netherlands*

The first TargetFish Industry Forum was held at the 16th International Conference of the European Association of Fish Pathologists (EAFP) held inTampere, Finland in September 2013. This "Industry Forum" was well-attended by representatives both the aquaculture and the aquatic animal health industries and provided networking opportunities between academic researchers and those working in the commercial sector.
The first TargetFish Industry Forum was held only a short while after the commencement of the TargetFish project and therefore performed the function of informing industry of the presence of the project and to provide more detailed information on the structure and membership of the consortium and details of the various work packages under the 'umbrella' of the Project.
The second Industry Forum which will be held on the 10th September as a workshop at the 17th International Conference of the EAFP will be three years into a five year project and at a time when a number significant findings crucial to the development of new vaccines and vaccine delivery systems have been made, and it is envisaged that the Industry Forum will encourage engagement between the academic researchers in the TargetFish project and Industry in the hope that the "batten" can be passed-on picked-up by the industry thus creating a smooth passage between the "research bench" and a commercial product and speeding-up the bringing of a new product to market and the improvement of existing products thus giving the TargetFish project considerable "impact". Such an approach should help to make EU-funded projects more-efficient in their outcomes and encourage industry engagement.
The industry co-ordinator for the TagetFish project is Tethys Aquaculture Ltd, an Impact Consultancy headed by Professor Patrick Smith.
More information the TargetFish 2nd Industry Forum can be obtained from Patrick Smith (patrick.tethysaquaculture@gmail.com) and details of the EAFP's 17th International Conference which will be held in Gran Canaria between the 6th and the 11th September can be found on the EAFP Website at www.eafp.org.

AUTHOR'S CONTACT

Abstract nº	Author	Email
	KEYNOTE PRESENTATIONS	
KN-1	B. Austin	brian.austin@stir.ac.uk
KN-2	A. Vanderplasschen	a.vdplasschen@ulg.ac.be
KN-3	A. Villalba	antonio.villalba.garcia@xunta.es
KN-4	P.J. Midtlyng	paul.midtlyng@aquamedic.no
	ORAL PRESENTATIONS	
O-001	J.C. Avarre	jean-christophe.avarre@ird.fr
O-002	J.C. Avarre	jean-christophe.avarre@ird.fr
O-003	J. Jarungsriapisit	jiraporn@imr.no
O-004	A. Nanjo	funyamo.az@gmail.com
O-005	T. Shibata	scat.ts215half@gmail.com
O-006	G. Wiegertjes	geert.wiegertjes@wur.nl
O-007	S.J. Harris	s.j.harris@keele.ac.uk
O-008	J.J. Miest	jmiest@geomar.de
O-009	S. LaPatra	scottl@clearsprings.com
O-010	F. Rivero	riverofer@yahoo.es
O-011	A. Saberi	afrasaberi@yahoo.com
O-012	I. Portugal	belcportugal@gmail.com
O-013	G. Paladini	giuseppe.paladini@stir.ac.uk
O-014	O. Palenzuela	oswaldo.palenzuela@csic.es
O-015	A. Repullés-Albelda	aigues.repulles@uv.es
O-016	J.Y. Zhang	zhangjy@ihb.ac.cn
O-017	P. Ronza	paolo.ronza@usc.es
O-018	D.G. Elliot	dgelliott@usgs.gov
O-019	L. Sandlund	liv.sandlund@uib.no
O-020	M. Adamek	mikolaj.adamek@tiho-hannover.de
O-021	G. Kato	gkato00@kaiyodai.ac.jp
O-022	H. Cordero	cordero@um.es
O-023	A. Rebl	rebl@fbn-dummerstorf.de
O-024	C.J. Chang	chia.j.chang@uit.no
O-025	D. Alvarez-Torres	dani-alto@uma.es
O-026	C.C. Norte dos Santos	catarina.nortedossantos@utas.edu.au
O-027	V. Emilsen	vibeke.emilsen@aquagen.no
O-028	L. Chalmers	lchalmers3@hotmail.co.uk
O-029	L. Andersen	linda.andersen@ilab.uib.no
O-030	B.E. Torstensen	bente.torstensen@nifes.no
O-031	D. Montero	daniel.montero@ulpgc.es
O-032	A. Sitjà-Bobadilla	ariadna.sitja@csic.es
O-033	D. Domínguez	david.dominguez103@alu.ulpgc.es
O-034	M.S. Izquierdo	marisol.izquierdo@ulpgc.es
O-035	S. Torrecillas	silvia.torrecillas@giaqua.org
O-036	J.A. García	gcabrera@vet.ucm.es
O-037	V. Kiron	kvi@uin.no
O-038	T. Sigholt	trygve.sigholt@biomar.no
O-039	J. Tinsley	jtinsley@biomar.co.uk
O-040	B. Petrujkić	petrujkic@vet.bg.ac.rs
O-041	H. Bleie	hogne.bleie@merck.com
O-042	S.R.M Jones	simon.jones@dfo-mpo.gc.ca
O-043	D.W. Kleingeld	dirk.kleingeld@laves.niedersachsen.de
O-044	M. Aldrin	magne.aldin@nr.no
O-045	A.B. Kristoffersen	anja.kristoffersen@vetinst.no

O-046	B. Bang Jensen	britt-bang.jensen@vetinst.no
O-047	H. Schűtze	heike.schuetze@fli.bund.de
O-048	R. Whittington	richard.whittington@sydney.edu.au
O-049	L. Jørgensen	lvgj@sund.ku.dk
O-050	J.K. Chettri	jkc@sund.ku.dk
O-051	G.T. Haugland	gyri.haugland@uib.no
O-052	T. Erkinharju	toni.erkinharju@uit.no
O-053	M. Nipkow	mareen.nipkow@gmail.com
O-054	S.R.M Jones	simon.jones@dfo-mpo.gc.ca
O-055	S. Monaghan	sjm27@stir.ac.uk
O-056	I. Cano	irene.canocejas@cefas.co.uk
O-057	S.C. Johnson	Stewart.Johnson@dfo-mpo.gc.ca
O-058	M.C. Gjessing	mona.gjessing@vetinst.no
O-059	C. Axén	marlene.areskog@sva.se
O-060	B. Gorgoglione	b.gorgoglione@vetmeduni.ac.at
O-061	T. Einarsdottir	thorbje@hi.is
O-062	M.E.J. Zorriehzahra	zorrieh@yahoo.com
O-063	I. Arzul	iarzul@ifremer.fr
O-064	S.A. Lynch	s.lynch@ucc.ie
O-065	A.L. Økland	arnfinn.okland@uib.no
O-066	R. van Aerle	ronny.vanaerle@cefas.co.uk
O-067	A. Özer	aozer@sinop.edu.tr
O-068	M. Ucko	mucko@ocean.org.il
O-069	V. Panzarin	vpanzarin@izsvenezie.it
O-070	S. Souto	sandra.souto@usc.es
O-071	S. Ciulli	sara.ciulli@unibo.it
O-072	A.M. Becker	anna.maria.becker@fau.de
O-073	M. Lund	morten.lund@vetinst.no
O-074	T. Taksdal	torunn.taksdal@vetinst.no
O-075	M. Dávidová	martinad@sci.muni.cz
O-076	T. Herath	t.k.herath@stir.ac.uk
O-077	M. Seifertová	seifertovamaria@gmail.com
O-078	L.M. Braden	lulu.braden@gmail.com
O-079	L.M. Braden	lulu.braden@gmail.com
O-080	C. McNair	c.m.mcnair@stir.ac.uk
O-081	A.A.A. Gamil	amr.gamil@nmbu.no
O-082	I. Arzul	iarzul@ifremer.fr
O-083	S.A. Lynch	s.lynch@ucc.ie
O-084	L. Madsen	loma@vet.dtu.dk
O-085	O. Gervais	ophelie.gervais@ifremer.fr
O-086	T. Bowden	timothy.bowden@maine.edu
O-087	N. Gagné	nellie.gagne@dfo-mpo.gc.ca
O-088	T. Aoki	takashi.aoki65@gmail.com
O-089	A. Rønneseth	anita.ronneseth@bio.uib.no
O-090	M.E.J. Zorriehzahra	zorrieh@yahoo.com
O-091	S. Gulla	snorre.gulla@vetinst.no
O-092	J. Frey	joachim.frey@vetsuisse.unibe.ch
O-093	S. Menanteau-Ledouble	simon.menanteau-ledouble@vetmeduni.ac.at
O-094	R. Thune	rthune@lsu.edu
O-095	C. Lang	catherine.lang@edqm.eu
O-096	Ø. Evensen	oystein.evensen@nmbu.no
O-097	B.R. LaFrentz	benjamin.lafrentz@ars.usda.gov
O-098	G. Guttormsdottir	gug70@hi.is
O-099	S. Bang Småge	sverre.smage@cermaq.com
O-100	Ø. Brevik	oyvind.brevik@cermaq.com

O-101	A.C. Barnes	a.barnes@uq.edu.au
O-102	T.J. Welch	tim.welch@ars.usda.gov
O-103	M.S.W. Breiland	mette.w.breiland@nofima.no
O-104	S.M. Bergmann	sven.bergmann@fli.bund.de
O-105	M.J. McGowan	mikel2564@gmail.com
O-106	S. Clouthier	sharon.clouthier@dfo-mpo.gc.ca
O-107	T. Morin	thierry.morin@anses.fr
O-108	M.J. McGowan	mikel2564@gmail.com
O-109	N.J.G Moody	nick.moody@csiro.au
O-110	M.E.J. Zorriehzahra	zorrieh@yahoo.com
O-111	P. Noguera	patricia.noguera@scotland.gsi.gov.uk
O-112	M. Danion	morgane.danion@anses.fr
O-113	M. Iannaccone	veterinariaesotici@gmail.com
O-114	M.D. Powell	mark.powell@niva.no
O-115	A.V. Kazarnikova	kazarnikova@gmail.com
O-116	B. Nowak	b.nowak@utas.edu.au
O-117	Ž. Trubić	ztrumbic@unist.hr
O-118	I. Mladineo	mladineo@izor.hr
O-119	I. Mladineo	mladineo@izor.hr
O-120	H. Stagg	hannah.stagg@scotland.gsi.gov.uk
O-121	R. Avendaño-Herrera	reavendano@yahoo.com
O-122	A. Barnes	a.barnes@uq.edu.au
O-123	F. El Aamri	fatima.elaamri@gmail.com
O-124	Z. Hanol Bektaş	zubeydehanol17@gmail.com
O-125	Y. Shimahara	shimahara@affrc.go.jp
O-126	P. Apablaza	patricia.apablaza@cermaq.com
O-127	T. Wahli	thomas.wahli@vetsuisse.unibe.ch
O-128	M. El-Matbouli	mansour.el-matbouli@vetmeduni.ac.at
O-129	N. Strepparava	nicole.strepparava@vetsuisse.unibe.ch
O-130	R. Paley	richard.paley@cefas.co.uk
O-131	M.D. Powell	mark.powell@niva.no
O-132	M. Marcos-López	mar.marcos.lopez@gmail.com
O-133	V. Valdenegro-Vega	victoria.valdenegro@utas.edu.au
O-134	A.M. Eriksson-Kallio	annamaria.eriksson-kallio@evira.fi
O-135	V. Jung-Schroers	verena.jung-schroers@tiho-hannover.de
O-136	D. Liu	liu@igb-berlin.de
O-137	K. Knopf	klaus.knopf@igb-berlin.de
O-138	N. Lorenzen	niels.lorenzen@anis.au.dk
O-139	N.J. Olesen	njol@vet.dtu.dk
O-140	Ø. Evensen	oystein.evensen@nmbu.no
O-141	H.F. Skall	hellef.skall@anis.au.dk
O-142	C. Joiner	claire.joiner@cefas.co.uk
O-143	H. Sigurðardóttir	heidasig@hi.is
O-144	H. Hauge	maria.dahle@vetinst.no
O-145	S. Zrnčić	zrncic@irb.hr
O-146	B. Nowak	b.nowak@utas.edu.au
O-147	C.R. Osorio	cr.osorio@usc.es
O-148	S. Hjartardottir	sigrhj@hi.is
O-149	B. Gorgoglione	b.gorgoglione@vetmeduni.ac.at
O-150	T. Hovland	tore.hovland@merck.com
O-151	K. Ulven	kristian.ulven@merck.com
O-152	A.M. Declercq	andclerc.declercq@ugent.be
O-153	L.B. Jensen	linda.jensen@skretting.com
O-154	V. Jung-Schroers	verena.jung-schroers@tiho-hannover.de

O-155	A. Klevan	are.klevan@pharmaq.no
O-156	E. Antuofermo	eantuofermo@uniss.it
O-157	I. Bušelić	buselic@izor.hr
O-158	J. Hrabar	jhrabar@unist.hr
O-159	M.J. Beckmann	r02mb12@abdn.ac.uk
O-160	J.O. Sunyer	sunyer@vet.upenn.edu
O-161	A. Sitjà-Bobadilla	ariadna.sitja@csic.es
O-162	T. Korytář	tkorytar@vet.upenn.edu
O-163	M. Ohtani	maki@sund.ku.dk
O-164	M.H. Marana	mmarana@sund.ku.dk
O-165	N. Kareem	n.o.kareem@keele.ac.uk
O-166	M. Galeotti	marco.galeotti@uniud.it
O-167	F. Acosta	felix.acosta@ulpgc.es
O-168	J. Bethke	jorn.bethke@gmail.com
O-169	F. Acosta	felix.acosta@ulpgc.es
O-170	J.G. Ramírez-Paredes	j.g.ramirezparedes@stir.ac.uk
O-171	E. Lewisch	eva.lewisch@vetmeduni.ac.at
O-172	C. Tafalla	tafalla@inia.es
O-173	K.A. Masterman	k.masterman@uq.edu.au
O-174	C.A. Shoemaker	craig.shoemaker@ars.usda.gov
O-175	M. Adamek	mikolaj.adamek@tiho-hannover.de
O-176	S. Gudmundsdotir	siggag@hi.is
O-177	A. Vanderplasschen	a.vdplasschen@ulg.ac.be
O-178	D. Sepúlveda	dagoberto.sepulveda@anis.au.dk
O-179	D.W. Verner-Jeffreys	david.verner-jeffreys@cefas.co.uk
O-180	D.L. Straus	dave.straus@ars.usda.gov
O-181	T. Meinelt	meinelt@igb-berlin.de
O-182	D.L. Straus	dave.straus@ars.usda.gov
O-183	R. Thorarinsson	ragnar.thorarinsson@novartis.com
O-184	D. Zilberg	dzilberg@bgu.ac.il
O-185	T.M. Pedersen	torbjorn@fom-as.no
O-186	S.S. Mikkelsen	susmi@vet.dtu.dk
O-187	E.J. Valverde	ejv@uma.es
O-188	M. Fourrier	mickael.fourrier@scotland.gsi.gov.uk
O-189	T. Wahli	thomas.wahli@vetsuisse.unibe.ch
O-190	R. Borzák	borzak.reka@agrar.mta.hu
O-191	C. Martenot	claire.martenot@ifremer.fr
O-192	I. Istiqomah	iendah_ist@yahoo.com
O-193	G. Ponte	g_ponte@cephalopodresearch.org
O-194	P. Tedesco	perla.tedesco2@gmail.com
O-195	V. Galligioni	viola.galligioni@gmail.com
O-196	P. García-Fernández	pablo_garcia05@hotmail.com
O-197	P. Tedesco	perla.tedesco2@gmail.com
O-198	S. Castellanos-Martínez	scastellanos@mda.cinvestav.mx
O-199	M. Solé	marta.sole@upc.edu
O-200	M. Petrić	mpetric@unist.hr

O-201	R.N. Grøntvedt	randi.grontvedt@vetinst.no
O-202	H.M. Munang'andu	hetroney.mweemba.munangandu@nmbu.no
O-203	J. Mewett	jason.mewett@cefas.co.uk
O-204	H. Schmidt-Posthaus	heike.schmidt@vetsuisse.unibe.ch
O-205	A.M. Declercq	andclerc.declercq@ugent.be
O-206	N.J.G. Moody	nick.moody@csiro.au
O-207	F. Pascoli	fpascoli@izsvenezie.it
O-208	S.C. Johnson	Stewart.Johnson@dfo-mpo.gc.ca
O-209	K. Veenstra	k.veenstra@abdn.ac.uk
O-210	S. Patel	sonal.patel@imr.no
O-211	S.L. Poynton	spoynton@jhmi.edu
O-212	M. Songe	m.songe@cgiar.org
O-213	M. Powell	mark.powell@niva.no
O-214	M.M. Solovyev	yarmak85@mail.ru
O-215	M. Podolska	bilbo@mir.gdynia.pl
O-216	R.M. Heavyside	rebecca.heavyside@skretting.com
	POSTER PRESENTATIONS	
P-001	H. Albayrak	harunalbayrak55@msn.com
P-002	H. Albayrak	harunalbayrak55@msn.com
P-003	T. Morin	thierry.morin@anses.fr
P-004	E. Blomkvist	eva.blomkvist@sva.se
P-005	D. Brnić	brnic@veinst.hr
P-006	E. Chaves-Pozo	elena.chaves@mu.ieo.es
P-007	A. Cuesta	alcuesta@um.es
P-008	A. Doszpoly	doszpoly.andor@agrar.mta.hu
P-009	A. Doszpoly	doszpoly.andor@agrar.mta.hu
P-010	C. Fritsvold	camilla.fritsvold@vetinst.no
P-011	J. Gu	jinni.gu@vetinst.no
P-012	P.H. Chang	penheng@ntu.edu.tw
P-013	V. Jenčič	vlasta.jencic@vf.uni-lj.si
P-014	V. Jung-Schroers	verena.jung-schroers@tiho-hannover.de
P-015	V. Jung-Schroers	verena.jung-schroers@tiho-hannover.de
P-016	A. Kydyrmanov	kydyrmanov@yandex.kz
P-017	D. Oraić	oraic@irb.hr
P-018	C. Ortega	cortegas@uaemex.mx
P-019	V. Panzarin	vpanzarin@izsvenezie.it
P-020	V. Piačková	piackova@frov.jcu.cz
P-021	M. Reichert	matthias.reichert@fau.de
P-022	M. Stachnik	magdalena.stachnik@piwet.pulawy.pl
P-023	M. Stachnik	magdalena.stachnik@piwet.pulawy.pl
P-024	M. Stachnik	magdalena.stachnik@piwet.pulawy.pl
P-025	M. Stachnik	magdalena.stachnik@piwet.pulawy.pl
P-026	R. Parreño	ricardo.parreno@graduado.umh.es
P-027	D. Castro	dcastro@uma.es
P-028	S. Ciulli	sara.ciulli@unibo.it
P-029	K. Way	keith.way@cefas.co.uk
P-030	M.E.J. Zorriehzahra	zorrieh@yahoo.com
P-031	M.E.J. Zorriehzahra	zorrieh@yahoo.com
P-032	M.E.J. Zorriehzahra	zorrieh@yahoo.com
P-033	M. Etyemez	metyemez@cu.edu.tr
P-034	A. Franke	afranke@geomar.de
P-035	M. Mancuso	monique.mancuso@iamc.cnr.it

P-036	N. Topić Popović	ntopic@irb.hr
P-037	A. Saraiva	amsaraiv@fc.up.pt
P-038	M.J. Beckmann	r02mb12@abdn.ac.uk
P-039	R. Chance	r01rc14@abdn.ac.uk
P-040	A. Fernández-Montero	alfer_90@hotmail.com
P-041	S. Hamilton	scott.hamilton@moredun.ac.uk
P-042	M.V. Rodrigues	mvazrodrigues@gmail.com
P-043	M. Ondračková	davidova_martina@yahoo.com
P-044	A. Özer	aozer@sinop.edu.tr
P-045	A. Özer	aozer@sinop.edu.tr
P-046	T. Öztürk	turkay.ozturk@gmail.com
P-047	T. Öztürk	turkay.ozturk@gmail.com
P-048	M. Palíková	palikovam@vfu.cz
P-049	T.M. Pedersen	torbjorn@fom-as.no
P-050	B. Repullés-Albelda	aigues.repulles@uv.es
P-051	M. Saleh	mona.saleh@vetmeduni.ac.at
P-052	B. Repullés-Albelda	aigues.repulles@uv.es
P-053	S. Tabakoğlu	saltinkaya@cu.edu.tr
P-054	B. Repullés-Albelda	aigues.repulles@uv.es
P-055	J. Wiik-Nielsen	jannicke.wiik@vetinst.no
P-056	M. Adamek	mikolaj.adamek@tiho-hannover.de
P-057	D. Alvarez-Torres	dani-alto@uma.es
P-058	L. Mercado	lmercado@ucv.cl
P-059	C. Bailey	christyn.bailey@vetsuisse.unibe.ch
P-060	R. Bermúdez	roberto.bermudez@usc.es
P-061	I. Cano	irene.canocejas@cefas.co.uk
P-062	E. Chaves-Pozo	elena.chaves@mu.ieo.es
P-063	S.C. Chen	scchen@mail.npust.edu.tw
P-064	L. Mercado	lmercado@ucv.cl
P-065	A. Cuesta	alcuesta@um.es
P-066	L. Mercado	lmercado@ucv.cl
P-067	Y. Kitani	yki@uin.no
P-068	B. Fouz	belen.fouz@uv.es
P-069	G.T. Haugland	gyri.haugland@uib.no
P-070	D. Haussman	dhaussmann@uach.cl
P-071	A.K. Siwicki	siwicki@uwm.edu.pl
P-072	S.D. Hwang	sdhwang@korea.kr
P-073	M.H. Jung	gloomy8983@hanmail.net
P-074	M.H. Jung	gloomy8983@hanmail.net
P-075	K. Kobayashi	k.kobayashi1206@gmail.com
P-076	H. Kondo	h-kondo@kaiyodai.ac.jp
P-077	C. Tafalla	tafalla@inia.es
P-078	D.L. Makrinos	daniel.makrinos@maine.edu
P-079	L. Mercado	lmercado@ucv.cl
P-080	I. Papežíková	papezikovai@vfu.cz
P-081	P.A. Santana	paula.santana@ucv.cl
P-082	P. Pereiro	patriciapereiro@iim.csic.es
P-083	P. Pereiro	patriciapereiro@iim.csic.es
P-084	P. Pereiro	patriciapereiro@iim.csic.es
P-085	M. Perelló	marta.perello@hipra.com
P-086	M. Perelló	marta.perello@hipra.com
P-087	L. Mercado P.A. Santana	lmercado@ucv.cl
P-088	S.D. Hwang	sdhwang@korea.kr
P-089	C. Valenzuela	valenzuela.i.cristian@gmail.com
P-090	J. Vega	judit.vega.alonso@gmail.com

P-091	C. Tzong-Yueh	ibcty@mail.ncku.edu.tw
P-092	S. Yoon	sohyeyoon@abdn.ac.uk
P-093	M.N. Yousaf	muhammad.yousaf@vetinst.no
P-094	A.M. de Azevedo	misabel.quiroga@usc.es
P-095	Y. Santos	mysabel.santos@gmail.com
P-096	J. Lokesh	loj@uin.no
P-097	J. Pajdak	joanna.pajdak@uwm.edu.pl
P-098	Ö. Diler	oznurdiler@sdu.edu.tr
P-099	M.A.G Owen	matthew.owen@skretting.com
P-100	M.M. Solovyev	yarmak85@mail.ru
P-101	M. Abbadi	mabbadi@izsvenezie.it
P-102	S.C. Bayliss	sb2145@bath.ac.uk
P-103	D. Jakić-Dimić	nivsd@yahoo.com
P-104	N. Diserens	nicolas.diserens@vetsuisse.unibe.ch
P-105	M.D. Jansen	mona-dverdal.jansen@vetinst.no
P-106	P.A. Jensen	peder.jansen@vetinst.no
P-107	S. Le Bouquin	sophie.lebouquin-leneveu@anses.fr
P-108	Z. Marková	zdena.13@seznam.cz
P-109	V. Radosavljević	vladimiradosavljevic@yahoo.co.uk
P-110	M. Seničar	marija.senicar@vf.uni-lj.si
P-111	T. Yatabe	tyatabe@ucdavis.edu
P-112	M.E.J. Zorriehzahra	zorrieh@yahoo.com
P-113	M. Constenla	maria.constenla@uab.cat
P-114	C.J. Francisco	clairejulianaf@yahoo.com.br
P-115	M.L. Fioravanti	marialeti.fioravanti@unibo.it
P-116	M. Galeotti	marco.galeotti@uniud.it
P-117	M. Areskog	marlene.areskog@sva.se
P-118	M. Marcos-López	mar.marcos.lopez@gmail.com
P-119	T. Pretto	tpretto@izsvenezie.it
P-120	F. Quaglio	francesco.quaglio@unipd.it
P-121	A. Vences	ana.vences@usc.es
P-122	T. Veselý	vesely@vri.cz
P-123	J. Wiik-Nielsen	jannicke.wiik@vetinst.no
P-124	M.J. Carballal	maria.jesus.carballal.duran@xunta.es
P-125	F. Carella	francesca.carella@unina.it
P-126	F. Carella	francesca.carella@unina.it
P-127	N. Carrasco	noelia.carrasco@irta.cat
P-128	F. Cervellione	fabio.cervellione@skretting.com
P-129	G. De Vico	gionata.devico@unina.it
P-130	S.A. Lynch	s.lynch@ucc.ie
P-131	T. Ryazanova	ryazanova.t.v@kamniro.ru
P-132	C. Lang	catherine.lang@edqm.eu
P-133	R. Cooney	a.tout@vmd.defra.gsi.gov.uk
P-134	M. Balado	miguel.balado@usc.es
P-135	S. Bartkova	sibar@vet.dtu.dk
P-136	T. Bossú	teresa.bossu@izslt.it
P-137	A. Cuesta	alcuesta@um.es
P-138	T. Baptista	teresa.baptista@ipleiria.pt
P-139	S.J. Harris	s.j.harris@keele.ac.uk
P-140	S.J. Harris	s.j.harris@keele.ac.uk
P-141	S.J. Harris	s.j.harris@keele.ac.uk
P-142	R. Avendaño-Herrera	ravendano@unab.cl
P-143	J. Figueroa	jefigueroa@uach.cl
P-144	J. Korun	jalekorun@akdeniz.edu.tr
P-145	S. Marozzi	selene.marozzi@izslt.it

P-146	C.A.G. Leal	carlosleal@vet.ufmg.br
P-147	S. Giskegjerde	siri@fom-as.no
P-148	A. Rønneseth	anita.ronneseth@bio.uib.no
P-149	B. Sellyei	sellyei.boglarka@agrar.mta.hu
P-150	T.S. Jung	jungts@gnu.ac.kr
P-151	A.K. Siwicki	siwicki@uwm.edu.pl
P-152	K. Varello	katia.varello@izsto.it
P-153	B. Sellyei	sellyei.boglarka@agrar.mta.hu
P-154	S. Viljamaa-Dirks	satu.viljamaa-dirks@evira.fi
P-155	J.H.Yu	kunydj@korea.kr
P-156	M. Adamek	mikolaj.adamek@tiho-hannover.de
P-157	K. Bateman	kelly.bateman@cefas.co.uk
P-158	J. Downes	jamie.downes@marine.ie
P-159	H.J. Kim	hjkim1882@korea.kr
P-160	O. Haenen	olga.haenen@wur.nl
P-161	C. Ortega	cortegas@uaemex.mx
P-162	P.F. Silva	polyana.silva@skretting.com
P-163	D. Vazquez	diego.vazquez@usc.es
P-164	D. Vazquez	diego.vazquez@usc.es
P-165	N. Vendramin	niven@vet.dtu.dk
P-166	S. Arana	aranauni@gmail.com
P-167	L. Baumann	lisa.baumann@vetsuisse.unibe.ch
P-168	A. Cuesta	alcuesta@um.es
P-169	V. Dobal-Amador	vdobal@uvigo.es
P-170	M. Danion	morgane.danion@anses.fr
P-171	N.Y. Kim	pharm001@korea.kr
P-172	M.G. Giacobbe	mariagrazia.giacobbe@iamc.cnr.it
P-173	A.K. Siwicki	siwicki@uwm.edu.pl
P-174	M.G. Giacobbe	mariagrazia.giacobbe@iamc.cnr.it
P-175	J. Řehulka	rehulka@szm.cz
P-176	G. Cech	cech.gabor@agrar.mta.hu
P-177	P.A. Smith	psmith@uchile.cl
P-178	T. Herath	t.k.herath@stir.ac.uk
P-179	Y. Kvach	yuriy.kvach@gmail.com
P-180	S. Arana	aranauni@gmail.com
P-181	M.A. Mahmoud	mahmoudaly@cu.edu.eg
P-182	S.J. Monaghan	s.j.monaghan@stir.ac.uk
P-183	G. Cech	cech.gabor@agrar.mta.hu
P-184	J. Naldoni	jnaldoni@gmail.com
P-185	J. Naldoni	jnaldoni@gmail.com
P-186	S. Özesen Çolak	aquasibel@hotmail.com
P-187	M. Polinas	mpolinas@uniss.it
P-188	M.I. Quiroga	misabel.quiroga@usc.es
P-189	M. Reichert	reichert@piwet.pulawy.pl
P-190	G. Rigos	grigos@ath.hcmr.gr
P-191	S.S. Bravo	sbravo@spm.uach.cl
P-192	T. Wahli	thomas.wahli@vetsuisse.unibe.ch
P-193	A. Saraiva	amsaraiv@fc.up.pt
P-194	E.A.V. Burioli	erika.burioli@izsto.it
P-195	E.A.V. Burioli	erika.burioli@izsto.it
P-196	Ch. Hebbar	racha_hebbar@hotmail.fr
P-197	O. Haenen	olga.haenen@wur.nl
P-198	I. Mladineo	mladineo@izor.hr
P-199	C. Cobo	cristobal.cobo@igb-berlin.de
P-200	A. Kubilay	aykub@yahoo.com

P-201	A. Kubilay	aykub@yahoo.com
P-202	B.I. Didinen	behiredidinen@hotmail.com
P-203	R. Hoare	rowena.hoare1@stir.ac.uk
P-204	A.V. Karzanikova	kazarnikova@gmail.com
P-205	J. Pajdak	joanna.pajdak@uwm.edu.pl
P-206	A.K. Siwicki	siwicki@uwm.edu.pl
P-207	S. Castellanos-Martínez	scastellanos@mda.cinvestav.mx
P-208	S. Castellanos-Martínez	scastellanos@mda.cinvestav.mx
P-209	C. Gestal	cgestal@iim.csic.es
P-210	C. Gestal	cgestal@iim.csic.es
P-211	I. Mladineo	mladineo@izor.hr
P-212	P. Tedesco	perla.tedesco2@gmail.com
P-213	A.E. Ahuir-Baraja	ana.ahuir@uv.es
P-214	L. Bigarré	laurent.bigarre@anses.fr
P-215	T. Hongslo	thorbjorn.hongslo@sva.se
P-216	M.A. Park	mapark@korea.kr
P-217	U. Al-Kindi	rawaa-2004@hotmail.com
P-218	S. Navrátil	navratils@vfu.cz
P-219	R. Sirri	rubina.sirri2@unibo.it
P-220	M.A. Tsai	p9416004@mail.npust.edu.tw