

**Monday September 3rd – Langevin / Cartier
Gill Health**

Moderator - Mark Powell (Institute of Marine Research - Norway)

3:15 PM	Gill Health	<u>Papanna</u> - Gill Diseases In Mediterranean mariculture: Factors That Exacerbate Gill Pathologies Under Fish Farming Conditions
3:30 PM		<u>English</u> - Possible Multi-Amoeba Aetiology of Amoebic Gill Disease (AGD) Of Farmed Atlantic Salmon
3:45 PM		<u>Powell</u> - Does Exposure To Cnideria Increase The Susceptibility Or Severity Of Amoebic Gill Disease In Atlantic Salmon?
4:00 PM		<u>De Jourdan</u> - Assessing The Avoidance And Preference Behavior Of Atlantic Salmon <i>Salmo salar</i> To Varying Oxygen Saturation Waters
4:15 PM		<u>Iqbal</u> - Parasitic Infection Of Red Tiger Oscar <i>Astronotus ocellatus</i> Imported Into Pakistan
4:30 PM		<u>Adamek</u> - When Hypnos Meets Thanatos - Physiological Impact Of A Carp Edema Virus Infection Of Common Carp Gills During Koi Sleepy Disease
4:45 PM		<u>Bright</u> - Histopathological Changes In The Gills And Skin of <i>Clarias gariepinus</i> Challenged With <i>Ichthyophthirius multifiliis</i> And Treated With Aqueous Leaves Extract of <i>Moringa oleifera</i>
5:00 PM		<u>Powell</u> - Functional Feed Ingredients Against Amoebic Gill Disease: Efficacy And Effects on Gill Inflammatory Responses



8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



Gill Diseases in Mediterranean Mariculture : Factors That Exacerbate Gill Pathologies Under Fish Farming Conditions

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Gills are the most delicate structures of fish that are in their simplest of structure, and in constant exposure to the environment they live-in. As a consequence of this they are easily pliable to the influences of the external environmental factors and internal pathophysiological alterations.

Gill Diseases caused by pathogenic and environmental agents are on the increase in the Mediterranean Marine fish farming in recent years. This presentation will highlight on the important gill diseases in Mediterranean marine aquaculture and the present control methods and limitations in executing suitable pathogen specific treatments.

The gill diseases were minimal in the beginning of Med aquaculture, however over the years various gill pathogens with specific pathological effects have appeared in the cultivated fish. Over the years the gill disease causing agents have also accumulated in the culture environments. With present environmental changes associated with global warming and constantly changing feeds due to the limitations on the availability of fish meal and increases reliance unsustainable plant protein quality in the present diets, fish are increasingly more susceptible to gill diseases and exhibit poor immune competence to cope with the stresses.

Conference Session Designation: (Gill Health)
Presentation Format: (Oral)



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Possible Multi-Amoeba Aetiology of Amoebic Gill Disease (AGD) of Farmed Atlantic Salmon

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Amoebic gill disease (AGD) is a parasite-mediated gill condition affecting many teleost fish globally, and it is the biggest health issue impacting farmed Atlantic salmon in Tasmania's expanding aquaculture industry. To date, *Neoparamoeba perurans* is considered the only aetiological agent of AGD, based on laboratory trials that confirmed its pathogenicity, and its frequent presence on the gills of infected farmed Atlantic salmon. However, the development of gill disease in salmonid aquaculture is complex and multifactorial, and is not always tightly associated with the presence of *N. perurans*. Moreover, multiple other amoeba species colonise the gills and their role in AGD is unknown. Previous reports of these accompanying amoebae on AGD-affected salmon based their taxonomic assessments on gross morphology alone, and are therefore likely inaccurate. The aim of this study was to more accurately document the diversity of amoebae colonising the gills of AGD-affected Atlantic salmon using a combination of morphological and sequence-based taxonomic methods. Amoebae isolated from AGD-affected salmon gills were characterised morphologically via light microscopy and transmission electron microscopy, and by phylogenetic analyses based on the 18S rRNA gene and COI gene. Apart from *N. perurans*, 11 other amoebozoans were found on the gills, and were classified within the genera *Neoparamoeba*, *Paramoeba*, *Vexillifera*, *Pseudoparamoeba*, *Vannella* and *Nolandella*. This comprehensive documentation of amoeba species highlights there is a far greater diversity of amoebae colonising AGD-affected gills than what is currently considered. Ongoing research which is investigating whether these accompanying amoebae are involved in AGD development, or can act as the primary agent in the absence of *N. perurans* will also be discussed.

Conference Session: (Gill Health)
Presentation Format: (Oral)
Student Presentation: (Yes)



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Does Exposure to Cnidaria Increase the Susceptibility or Severity of Amoebic Gill Disease in Atlantic Salmon ?

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Amoebic gill disease (AGD) is a significant disease affecting Atlantic salmon, *Salmo salar* aquaculture in Europe, North America, and Australia and is often a component of what is now referred to as “complex gill disease” – a multifactorial response to multiple potentially gill pathology causing agents. Of these potential agents, cnidarians (hydroids and scyphozoa) are known to cause gill pathology alone where contact with the nematocysts has the potential to cause irritation and pathological damage to salmon gills. This study examined the potential implications of pre-exposure of Atlantic salmon (*Salmo salar* L). smolts to sublethal levels of hydrozoa (*Ectopleura larynx*) or scyphozoan jellyfish (*Cyanea capillata*) 24h prior to challenge with *Neoparamoeba perurans*, the causative agent of AGD. This study used laboratory challenge trials to: (1) characterise the gill pathology resulting from the exposure of salmon to *E. larynx* or *C. capillata*, and (2) investigate if such exposure can predispose the fish to secondary infection – using *N. perurans* causing amoebic gill disease (AGD). Gill health (AGD gill scores, non-specific gill scores, lamellar thrombi, epithelial hyperplasia) was monitored over 5 weeks and compared to an untreated control group. In both cases, higher average numbers of gill lamellar thrombi occurred in fish up to 7 days after exposure to hydroids or jellyfish. However, gill pathologies caused by hydroids did not affect the infection rates of *N. perurans* or the disease progression of AGD based upon gross gill score or histopathology. On the other hand, pre-exposure to jellyfish appeared to retard the development of AGD over the initial 3 weeks post-challenge based upon gross gill score. Thereafter, gross gills scores between *N. perurans* only and *C. capillata* and *N. perurans* combined were equivocal. Similarly, the prevalence of *N. perurans* positive fish (based upon qPCR analysis of *N. perurans* mRNA) indicated that there were fewer amoebae on the gills of fish in the combined challenge, compared with those of the *N. perurans* only group. This study indicated that cnidarian pre-exposure prior to infection with *N. perurans* did not enhance the rate of infection, not severity of disease and potentially may have lessened the impact of infection. The reasons for this are not clearly understood but may reflect the nature of the inflammatory responses occurring in the gills in response to cnidarian envenomation with nematotoxins.

Conference Session Designation:

(Gill Health)

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Assessing the Avoidance and Preference Behaviour of Atlantic Salmon *Salmo Salar* to Varying Oxygen Saturation Waters

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Temperature has a significant role in governing the metabolic rate in poikilotherms like fish, with increases in temperature corresponding to increases in metabolic rate and subsequently oxygen demand. Ironically, increases in temperature also lowers the solubility of oxygen, thereby reducing the supply of dissolved oxygen available for respiration. These two intrinsically linked water quality parameters have a major impact on the ecology of aquatic species, and it is not uncommon for them to be negatively affected in the presence of hydroelectric dams. This may, in turn, negatively impact migration and result in lower returns of culturally and economically important fish species.

This study was designed to provide behaviour data of Atlantic salmon (*Salmo salar*) as it relates to avoidance or preference in a two-current flume choice system with waters of varying oxygen saturation. Study water was made up on demand using a proprietary gas infusion system to infuse either oxygen or nitrogen to raise or lower the measured dissolved oxygen concentrations, respectively, while maintaining water total gas pressure. A total of 27 trials were conducted with groups of 10 fish (total = 270 fish) tested in a two-current choice flume. Each current (left and right) had varying concentrations of dissolved oxygen (ranging from 68 to 125% saturation). The trials were conducted at three different temperatures (8, 10, and 12°C) and involved nine combinations of flumes, for a total of 81 individual runs. Each run was 10 minutes in duration and was recorded by an overhead GoPro Hero3+ camera. Video analysis of each run was performed using ImageJ and ToxTrac software. From the video analysis, gross and net avoidance were calculated for each group of fish relative to oxygen saturation, and swimming performance metrics (e.g., swimming speed, acceleration, and exploration rate) were determined in each treatment flume.

While the data did not support a preference or avoidance related to freshwater oxygen saturation within the tested concentrations, we noted significant increases in swimming speed and acceleration with increasing concentrations of dissolved oxygen. These behaviours, and the benefits of higher oxygen saturated freshwater, may be more important to fish ladder performance compared with fish preference. Greater swimming capacity displayed within high oxygen saturation may enhance fish abilities to cross ladders and move past dams during their incoming annual migration. Discussion of these laboratory results will be augmented with data from ongoing field trials involving the deployment of this innovative technology in a fish ladder at a hydroelectric dam.

Conference Session Designation: (Climate Change & Aquatic Animal Health)
Presentation Format: (Oral)



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Parasitic Infection of Red Tiger Oscar, *Astronotus ocellatus* Imported into Pakistan

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The aim of present study was to observe parasitic infection in red tiger Oscar, *Astronotus ocellatus*, an ornamental fish imported into Pakistan. Total 30 specimens of red tiger oscar, were obtained from a pet shop in Lahore and were examined clinically and histopathologically. The mean total length and mean body weight of the fish were 7.82 ± 2.99 cm and 8.59 ± 6.07 g respectively. Clinically infected fish showed eroded dorsal and caudal fins. One fish did not have left eye ball and two fish had curved vertebral column. One fish had white spot on the body. Skin, fins and gills were observed under microscope by wet mount preparation. Gills were also examined histologically for detailed observations.

A total of 7,503 parasites were recorded in red tiger oscar fish. The parasites observed were protozoans; *Ichthyophthirius multifiliis* (13.3%, MI=257) and *Piscinoodinium pillulare* (10.0% MI=480.6); monogeneans, *Dactylogyrus* sp. (96.66%, MI=139.3), *Gyrodactylus* sp. (10%; MI=196.6); digeneans; *Postodiplostomum* sp.(6.66%; 151.5) and encysted metacercaria of trematode (6.66%; 49.5). Gill infection by *Dactylogyrus* sp. was the most prevalent compared to infection by other parasites. Histological results of infected gills showed; hyperplasia, fusion of secondary lamellae, swollen nodules on tips of gill filaments as a result of attachment of *Dactylogyrus* sp. and *Ichthyobodo* sp. on the gills. Gill infection may be categorized as low, mild and severe. Large fish showed low infection as compared to small fish. The present observations raise issues with regard to veterinary inspection of the imported fish. Bio-security measures including strict quarantine must be adopted to avoid transmission of parasites into local fish fauna of Pakistan.

Conference Session Designation: (Gill Health or Ornamental Fish Diseases)

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When Hypnos Meets Thanatos - Physiological Impact of a Carp Edema Virus Infection of Common Carp Gills During Koi Sleepy Disease

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Koi sleepy disease, caused by an infection with carp edema virus is a unique virus model which predominantly affects carp gills. Initially infected fish are lethargic, start laying at the bottom of the tank. With the progress of the disease, the activity of the fish decreases until nearly complete stillness, followed by death. We hypothesized that these clinical signs are related to gill dysfunction. Therefore, the pathophysiological impact of the infection was measured, including an analysis of the respiration, ammonia excretion and the hydro-mineral balance. Furthermore the blood plasma metabolome of KSD affected fish was studied. The experimental setup included two strains of carp (AS and koi) with different susceptibilities to KSD. All carp were cohabitated with koi infected with a CEV variant from genogroup IIa. During four infection experiments performed at 18 °C 100% of the koi developed severe KSD, which led to a complete immobilisation of the animals at the bottom of the tank between days 6 and 12 post infection (p.i.) with a peak at days 6 p.i.. In blood collected at days 6 and 9 p.i., the oxygen content was slightly reduced, sodium and calcium concentration extremely decreased (e.g. Na⁺ dropped from 130 mmol l⁻¹ in control to 82 mmol l⁻¹ in infected group), and ammonia levels severely increased from 212 µmol l⁻¹ in controls to 658 µmol l⁻¹ in infected fish. Analyses of over 2,500 metabolites showed changes in the pyrimidine and urea cycle as well as the beta-alanine and amino acid metabolism in blood plasma at day 6 p.i.. These changes occurred only in clinically affected koi while clinically healthy AS strain carp remained unaffected. This correlated with a much higher virus load and the onset of histopathological changes in the gills of koi. Furthermore, a 0.6% NaCl supplementation to keeping water was able to prevent the fish from developing clinical signs of KSD including the sleepy behaviour, the elevated ammonia level and the loss of ions measured in blood. The bath however, did not stop the virus infection and virus load did not differ between fish kept on the NaCl and not supplemented groups. Taken together the results suggest that the sleepiness of the fish is not related a lack of oxygen but to a disruption of the waste removal from the amino acid metabolism which leads to intoxication with ammonia. While the death is most likely caused by the severe osmotic imbalance.

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Histopathological Changes in the Gills and Skin of *Clarias gariepinus* Challenged with *Ichthyophthirius multifiliis* and Treated in Dip Bath Treatment with Aqueous Leaves Extract of *Moringa oleifera*

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Ichthyophthirius multifiliis (Ich) is a ciliate protozoan pathogenic parasite in the wild and freshwater system that parasitizes gills and skin of freshwater fish, *Clarias gariepinus*. The use of chemotherapeutants has promoted residual chemical drugs on the body of the fish, therefore the need for the use of environmental friendly herbal therapy has a great potential as a suitable replacement for chemotherapeutants and a good pharmacopeia in aquaculture. A study was conducted to investigate the effects of aqueous leaves extract of *Moringa oleifera* in the histology of the skin and gill of *Clarias gariepinus* challenged with *Ichthyophthirius multifiliis*. Six concentrations of aqueous leaves extract of *Moringa oleifera* were exposed to ich-infested fish for 1h to limit the impact of the adult parasite (trophont) in juveniles of *Clarias gariepinus*. The cumulative incidence of the Ich infestation was significantly lowered in the skin and gill of the treated fish compared to the negative control ($p < 0.05$). The major histopathological alterations revealed abnormal and some significant morphology characteristics in the skin; fatty degeneration, abscess formation, degeneration of the muscle fibers while presence of matured adult parasite embedded on the gill lamellae, degenerated secondary lamellae, edema and epitheliocystis were observed in the gill. An irreversible lesion was observed in the skin and gill of the negative control. Meanwhile, the overall lesion scores analyzed using kruskal wallis revealed asymptotically significant changes; oedema ($p = 0.041$), severe destruction of the secondary lamellae ($p = 0.025$), fatty degeneration ($p = 0.041$) and inflammatory infiltrates ($p = 0.02$) to be the most observed damage among the groups before the treatment began. Ich parasitic infestations in the organs of *C. gariepinus* are very dangerous due to observed lesions that are putative routes for secondary infection and subsequent manifestation of diseases. These results indicate that the use of aqueous leaves extract of *Moringa oleifera* reduced the adult parasite in the gills and skin of Ich-infested fish, although with clear tissue damage but cannot be ruled out as an environmental friendly herbal therapy for controlling ichthyophthiriasis.

Conference Session Designation: (Aquatic Animal Health Management)
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Functional Feed Ingredients Against Amoebic Gill Disease: Efficacy and Effects on Gill Inflammatory Responses

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Amoebic Gill Disease (AGD) is rapidly becoming a significant health issue in Norwegian fish farming. The causative agent, *Neoparamoeba perurans* is often seen as a single agent disease, AGD is also observed as a component of a complex of potential gill pathogens resulting in a multifactorial gill disease. The primary treatment of the disease has only two commercially available options; freshwater and hydrogen peroxide. Investigation of potential functional feed candidates to be used to slow disease progression and form part of an integrated pathogen management program has been undertaken. Firstly, an assay for investigating the efficacy of a feed candidate was developed for screening potential candidate compounds supplied by Cargill innovation. After the screening process, three candidates was picked for in-vivo direct challenge pilot trials and incorporated into test diets. A strong correlation between results of in-vitro screening, and in-vivo challenge trials, based on gill scores and RT-PCR for *Neoparamoeba perurans*. Pilot testing of two of the potential candidate compounds showed significant reductions in gross gill pathology and histological lesions. For one compound, gill gene expression was examined and showed significant changes supporting the development of gill inflammation and suppression of epithelial cell hyperplasia consistent with the histological findings. These results suggest that functional dietary feed ingredients against *Neoparamoeba perurans* can also modulation of gill inflammatory processes.

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