Thursday, September 6th – MacDonald
QASH 1 & 2
Moderators – Karin Pittman, Mark Powell, Robin Shields & Linda Andersen

9:30 AM	**Welcome to QASH – The QASH Core Team**
Biomarkers Session - Karin Pittman (Moderator)	
9:35 AM	Powell - A Healthy Fish Can Handle What Nature Throws At It: Allostasis In Fish Health
9:50 AM	Braceland - Challenges In The Biomarker Pipeline
10:05 AM	Gutiérrez - A Risk Assesment Matrix For Smolt Welfare In Atlantic Salmon: Insights From Chile
10:20 AM	Auchterlonie Declining Marine Ingredient Inclusion Levels And A Hypothesized Link With Fish Health In Farmed Atlantic Salmon
Barriers and stressors session - Mark Powell (Moderator)	
10:35 AM	Pittman - Barrier Status In Skin, Gills And Guts: Mapping The Dynamics Of The Innate Immune System Throughout The Production Cycle With Statistically Robust Results
10:50 AM	Chikwati - Gut Health Monitoring During The Seawater Phase Of Farmed Atlantic Salmon In Different Produciton Regions Of Norway - The GutMatters Project
11:05 AM	Mella - Practical Applications of Quantitative Image-Based Assesment Of Digital Pathology Slides In Chilean Salmon Industry
11:20 AM	Sveen - Wound Healing And the Effect Of Chronic Stress In Post-Smolt Atlantic Salmon (*Salmo salar*)
11:35 AM	**Workshop 1 - Biomarkers and barriers - criteria, cutoff levels, long-term effects, remedial actions? - Mark Powell and Karin Pittman**

A Healthy Fish Can Handle What Nature Throws at it: Allostasis in Fish Health

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Determining the criteria for what is a healthy fish poses many challenges and alternate definitions. Given that health is not merely the absence of disease, that an infected fish is not a diseased fish, it is important to look at the physiological capacity of a fish to respond to a wide range of biological and environmental challenges. The concept of allostatic load is one in which an organism can respond to a range of challenges within an allostatic range – a range of adaptation and tolerance. However, once a threshold is reached, the response goes from being one of adaptive to maladaptive crossing over the patho-physiological limit. In response to infectious and non-infectious challenges, determination of the pathophysiological threshold is difficult. The application of current studies using clinical chemistry and histopathological responses to infectious and non-infectious disease, environmental and management challenges in salmon highlights the plasticity of fish patho-physiological processes. Using current evaluation techniques for gill responses, gill pathophysiology and histopathology we examine whether the cure can sometimes be worse than the disease and how compounding effects of treatments can compromise a fish that is in a state beyond the pathophysiological threshold.

Conference Session Designation: (QASH)
Presentation Format: **(** Oral)

Challenges in the Biomarker Pipeline

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Defining, and our abilities to do so, healthy populations is one of the greatest challenges faced to livestock industries generally. As such a multitude of scientific literature aims to establish biomarkers of infection, infestation, sub-clinical, clinical, and projected disease outcomes. However, few of these candidate biomarkers establish in a clinical setting. The pipeline of development includes several stages from discovery to implementation. However, due to the failures and issues with each of these few pass from discovery to implementation. Furthermore, the ability of a marker to pass through this pipeline is often blocked due to pre-conceived notions and the entrenchment of established practices which may not be appropriate and/ or better than that in development. This presentation aims to exemplify the process of a biomarker's discovery, validation, translation, evaluation, and implementation with the pitfalls of these steps being explained.

Conference Session Designation: (Quantitative Atlantic Salmon Health)
Presentation Format: (Oral)

A Risk Assessment Matrix for Smolt Welfare in Atlantic Salmon: Insights from Chile

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Salmon farming productivity strongly relies on smolt adaptability success on seawater (SW) environment after transference from the freshwater (FW) phase, especially during the first weeks after the arrival. According to current understanding, sub-optimal water quality conditions at tank level are able to alter key smolt physiological traits (e.g osmoregulation) which can be critical for fish growth and survival. Even though important advances on smolt welfare from land-based farm exist, there is a lack of quantitative tools able to better link fish physiological traits with SW smolt performance (e.g smolt index). A risk assessment on key water quality parameters, as well as smolt physiological indicators has been proposed as a first step towards a physiological smolt welfare index in Chile.

The current study is based on data from an ongoing smolt physiological monitoring program undertaken by NIVA Chile from both RAS-based and flow-through (FT) fish farms since 2015. The risk assessment is based on three components: key water quality, blood parameters and metals accumulated in target organs (gills and liver). A total of 20 consecutive batches of smolts were examined under these components only days before the smolts are transferred to the sea farms. Batch sampling was based on 3 tanks in which water quality parameters from effluents were analyzed. For each tank, 6 individuals were sampled to measure blood parameters, from which 3 individuals were randomly chosen to collect gills and liver samples.

Using the database of previous projects conducted in fresh water salmon farms, in conjunction with revising scientific literature, limits and recommended levels were established for all components considered. This enabled the categorization of each parameter by providing ranges in which they represent low, medium, or high implications on fish welfare. The frequency (or probability of occurrence) at which each variable presented low, medium or high implications was also determined. The combination of implication/severity level and frequency level results in a qualitative matrix for risk assessment considering the most critical variables.

This matrix appears as a suitable tool for visualizing the main risks for smolt welfare depending on production system (RAS or FT). Outcomes from this matrix can serve as a guideline for decision making process to correct and minimize the risk of these variables. The producer, for example, is able to prioritize and improve aspects of water quality conditions which in turn, result in better fish welfare.

Conference Session Designation: **(** Quantitative Atlantic Salmon Health)
Presentation Format: **(** Oral)

Declining Marine Ingredient Inclusion Levels and an Hypothesised Link with Fish Health in Farmed Atlantic Salmon

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The decline in inclusion rate s for fishmeal and fish oil is well understood, occurring since the late 1990s for salmon feed in particular, but across all the major farmed fish species groups and their formulations. The current situation is a consequence of both supply volume, and price, and the feed companies need to develop aquafeed volume supply to meet growing demand over time. Annual fishmeal and fish oil production is finite and additional feed volume has come from other ingredients, notably vegetable-based materials such as soya and wheat, of necessity.

The story is one of supplementation as the marine ingredients continue to be the foundation for aqafeeds but in much decreased concentrations in comparison to the feeds that were used in the early years of the modern aquaculture industry. In order to achieve effective substitution of marine ingredients in diets feed companies invested heavily in research in order to ensure that growth performance has not been impacted by changing raw materials use. One aspect of the reduction of fishmeal in particular is the change in supply across the micronutrients that are known to be found in rich concentrations in fishmeal, and which are not found in other protein sources to the same extent. In that respect, the minerals such as Fe, Ca, Zn, Se are important as well as the B-group vitamins and vitamin D, all possibly playing a role in immunocompetence and the ability to cope with pathogen challenge. In some respects the possible impact on fish health of the reduction in supply of these materials is unknown with traditional deficiency studies focused on meeting minimum requirements rather than optimal levels. There also exists the question of how feed composition may influence the gut microbiome, and the link that may have with fish health. Improving farmed salmon's ability to cope with pathogen challenge has the potential to improve production efficiencies.

The situation will vary for different species and production systems, and will certainly be very complex, but it is important to know the full impact that substitution and supplementation has had, and its impact on fish health. The Atlantic salmon as a species is an excellent model to look at these impacts in the first instance.

Conference Session Designation: (Quantitative Atlantic Salmon Health)
Presentation Format: (Oral)

Barrier Status in Skin, Gills and Guts: Mapping the Dynamics of the Innate Immune System Throughout the Production Cycle with Statistically Robust Results

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Mucosal barriers of skin, gills and guts are the primary tissues protecting the fish from outside challenges like pathogens and parasites and the internal challenges of suboptimal feeds. The mean size and volumetric density of mucous cells producing this protective slime were first quantifiable in 2010 (Pittman et al. 2011, 2013) and the technique has since been applied in over 50 large and small scale trials in 6 countries, 6 species and 3 tissues with subdivisions. Now trademarked as Veribarr, the verification of the living barriers, results show that there is a reproducible "behaviour" from these tissues in response to a variety of inputs and as such may indicate "herd health" in addition to reflecting individual status. Mean cell size, volume of mucous in the skin epithelium and the combination of these factors shows how each tissue responds both in concert with the others and independently in response to eg. diet. The growing database allows the ascertainment of normal ranges for each tissue and species, while the objective measures allow comparison across species, time, treatment and tissue. Currently two projects are exploring the link between microarray data, RNA analyses and Veribarr results to look for reliable markers of healthy homeostasis in skin or guts. Results further show that a sufficiently sized non-lethal gill biopsy will give rise to reproducible results and may be used to possibly indicate general health. The method is complementary to all other existing methods investigating fish health.

Conference Session Designation: (QASH)
Presentation Format: (Oral)

Gut Health Monitoring During the Seawater Phase of Farmed Atlantic Salmon in Different Production Regions of Norway – The Gutmatters Project

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Optimization of feed resource utilization, production costs, fish growth performance, and environmental impact of Atlantic salmon industrial production is highly dependent on a healthy, optimally functioning gut. Overt clinical signs of gut dysfunction are rare in field conditions, yet subclinical gut health disorders can significantly diminish fish performance and health. Regardless, gut health of farmed fish populations at sea is not routinely monitored for impacts of feed, noxious, infectious or parasitic agents. As part of the ongoing GutMatters project funded by the Norwegian Seafood Research Fund, a national survey to establish the prevalence of gut health disorders and their incidence during a production cycle in sea farmed Atlantic salmon was therefore initiated in the autumn of 2017. Six sea farming sites along the Norwegian coast were monitored starting at about 5 weeks after sea-transfer until about 12 months of the fish at sea. Standardized procedures were developed for comprehensive sampling of up to 20 fish per site, including external and abdominal gross pathology, fish weight, length and blood plasma, content and tissue from intestine, liver, head kidney, spleen, and heart for histology and/or gene expression, microbiota, metabolomics, and digestive enzyme activity analyses. At each of 3 sampling events per farm, site physico-chemical data, fish stock feeding, growth, and health history were collected. Histology was used as the initial screening tool for gut health status of the sampled fish from which subsequent analyses will be based on. This presentation reports results from a semi-quantitative histology scoring for selected inflammatory and degenerative morphological changes in the mucosa of the pyloric caeca, mid-, and distal-intestine and the liver. Main findings from the histological evaluation of the fish sampled after 5 weeks post sea-transfer were mild to moderate inflammatory changes in the distal intestine of most of the fish sampled from one of the participating farms, as well as mild to marked enterocyte steatosis in the pyloric caeca in most of the groups evaluated. The inflammation resembled the well-documented soybean meal induced distal intestinal enteritis observed in salmonids fed diets containing soybean, or other legumes, as a protein source. The steatosis is thought to represent a lipid transport or metabolism disorder in enterocytes that in severe cases manifest as lipid malabsorption, steatorrhea and 'floating faeces'. Both disorders of inflammation and steatosis may markedly diminish gut function, fish growth and health and are candidate indicators of reduced feed utilization in Atlantic salmon. Details from the histological assessment of the gut and liver from fish sampled during the first and second round of monitoring the participating sea farms will be presented.

Conference Session Designation: **(** Quantitative Atlantic Salmon Health or Nutrition)
Presentation Format: (Oral)

Practical Applications of Quantitative Image-Based Assessment of Digital Pathology Slides in Chilean Salmon Industry

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Pathology diagnosis has been performed by pathologists observing the stained specimen on the slide glass using a microscope. In recent years, attempts have been made to capture the entire slide with a scanner and save it as a digital image (Whole slide image, WSI). Researchers both in the image analysis and pathology fields have recognized the importance of quantitative analysis of pathology images. Since most current pathology diagnosis is based on the subjective (but educated) opinion of pathologists, there is clearly a need for quantitative image-based assessment of digital pathology slides. In VeHiCe we adopt these analyses to be applied in farmed salmon industry in Chile. Currently this method is applying to assess pathologies evolution, health status, organs responses to drugs and diets among others. Allowing farmers take objectives measures regarding health and productions issues.

This study reports the assessment of the effects of anti-inflammatory drug in the evolution of an inflammatory process in the heart fibers caused by PRV. Two groups were tested; control group and T1. In total, the heart of 50 fish with were histologically processed, transverse sections of the ventricle were made and then the whole histopathological slide was capture with scanner and save as a digital image (WSI). The 85%-95% of total surface of the ventricle was analyzed in each slide. The morphometric analyze of the images was realized using ImageJ v1.49 (National Institut of Health, EE.UU.). In total 550 images were analyzed. In control group, there was 6,18% of the total heart surface presenting an inflammatory process, and in T1 group only a 0,47% of the total heart surface presented inflammation.

Using this method, we were able to measure precisely the % of inflammation in the different groups analyzed and consequently the evolution of the inflammatory process and the response to the tested drug.

Conference Session Designation: (Quantitative Atlantic Salmon Health)
Presentation Format: (Oral)

Wound Healing and the Effect of Chronic Stress in Post-Smolt Atlantic Salmon (*Salmo Salar*) **(WITHDRAWN)**

Lene Rydal Sveen [a,b,*], Gerrit Timmerhaus[b], Aleksei Krasnov, Harald Takle[d], Sigurd Handeland[c], Sigurd Stefansson[a], Elisabeth Ytteborg[b].

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The skin of Atlantic salmon (Salmo salar), is a coherent and dense barrier that protects the interior of the fish against the outer environment. The skin covers the entire outer surface, including the head, fins and eyes. Lesions in the skin are a major welfare issue for the fish.

Atlantic salmon post-smolts (mean weight 120g) were divided into two identical tanks (500L), and two treatments were established. High production intensity, HPI (mean fish density 126 kg/m^3) and normal production intensity, NPI (mean fish density 16 kg/m^3) . Three cylinders of tissue were excised with a 5 mm biopsy punch. Samples (n=12 per treatment) for gene expression analyses (microarray), histology, immunohistochemistry and scanning electron microscopy were collected 1, 3, 7, 14, 36, 42 and 57 days post wounding (dpw).

In general, the wounds from both HPI and NPI followed the normal progression of wound healing, with hemostasis, re-epithelialization, inflammation, tissue formation and tissue remodeling. The first 14 days of the healing process was dominated by acute inflammation and epidermal repair as shown through imaging, histological evaluation and transcriptomics. In the early inflammatory phase a more adherent mucus layer was observed, which further correlated with altered transcription of glycosyl transferases and mucin genes. This may indicate different properties and functions of the mucus during the acute inflammatory phase. Formation of scales and granulation tissue started approximately at 14 days post wounding. This was followed by wound contraction and formation of dermal structures.

At the transcriptomic level the greatest differences between NPI and HPI were found at 2-14 dpw, with more than > 500 DEG at each sampling point. In general, inflammation was enhanced in the HPI wounds, while cell proliferation and tissue regeneration was repressed. Histological examinations showed transient delays in the formation of epidermis, mucus response, scale mineralization, wound pigmentation and formation of dense connective tissue in HPI wounds. The overall wound morphology was also altered in fish reared at HPI. Wounds from NPI fish contracted in an elongated manner, while the wounds from HPI fish were more circular. The fish reared at HPI had significant higher cortisol levels compared to NPI fish, thus we suggest that cortisol are one of the main factors contributing to the delayed wound healing responses in fish reared at HPI .

The presented description of the wound healing processes in Atlantic salmon and the effect of HPI, gives insight into comparative ulcerative biology in fish and provides both novel and updated knowledge that can be applied for improved best operational practices for fish welfare in aquaculture.

Conference Session Designation: (QASH)
Presentation Format: (Oral)