

Tissue Barriers



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Carlo C. Lazado

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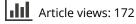
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The 1st international symposium on mucosal health in aquaculture – MHA2019

Carlo C. Lazado 💿

Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, Ås, Norway

ABSTRACT

The 1st International Symposium on Mucosal Health and Aquaculture (MHA2019) was held on 11-13 September 2019 in Oslo, Norway. This was the first platform of its kind that gathered people from academia, R&D institutes, and industry to discuss the state-of-the-art and future directions of mucosal health research in aquaculture. The symposium was divided into four scientific sessions: Session 1, Mucosal structures and functions; Session 2, Mucosal health and nutrition; Session 3, Mucosal health and microbiome; and Session 4, Mucosal health and the changing environment. A plenary talk from a prominent authority in the field opened each session. The papers presented at MHA2019 exemplified the dynamic evolution of the field of mucosal health in aquaculture - from an area solely explored from an immunological aspect about 20 years ago, to the multidisciplinary field it is today. Moreover, papers presented highlighted the complementary application of both classical (e.g., histology) and modern platforms (e.g., omics, artificial intelligence) to characterize mucosal health. The interactive discussion during the meeting underscored the importance of mucosal health research in modern aquaculture and collectively emphasized the role of both fundamental and applied approaches in advancing this timely and highly relevant field. The symposium was organized by Nofima, The Norwegian Institute of Food, Fisheries, and Aquaculture Research, with support from the Research Council of Norway.

1. Mucosal barriers of fish

Fish have an intimate interaction with their immediate environment, where several challenges present themselves, including biological (e.g., parasites, bacteria, viruses), chemical (e.g., pollutants, therapeutics), and physical (e.g., handling sorting in commercial production).¹ These challenges put significant pressure on the adaptive and protective strategies of fish. The gills, skin, gut, and nasal mucosa are the major mucosal organs in fish, and they represent the interface between the internal and the external environments. Mucosal barriers are emblematic components of these organs and include a repertoire of physical and biological defenses - the mucus layer, microbiota, and tissue structure, all acting in tight coordination to ensure the fidelity of barrier functionality.² They are often referred to as the first line of defense in fish. Moreover, they respond well to environmental changes, hence the moniker "the biological sensors", which have been exploited to provide readouts about the quality of the captive environment.

2. MHA2019 - the first of its kind

Producing healthy and robust fish is a central aspect of modern aquaculture. Besides being able to resist infection pressures, the ability to mount responses and cope with challenging conditions is a characteristic that is valued. In the last 15 years, intensive research has been dedicated to understanding the physiology and immunology of mucosal barriers in fish. The relevance of mucosal health for aquaculture is supported by the fact that the health status of the mucosa is reflected in the overall health status of fish, resulting in the development of several strategies targeting the mucosal surfaces for improved health of farmed fish. Despite the unequivocal importance and global interest on mucosal health in aquaculture, there is no dedicated platform for this theme in aquaculture. MHA2019 was conceptualized to address this. The 1st International Symposium on Mucosal Health in Aquaculture (MHA2019) was the first meeting of its kind and was held on September 11-13, 2019 in Thon Hotel Storo in Oslo, Norway.³ The meeting aimed to provide a platform for researchers,

CONTACT Carlo C. Lazado 🖾 carlo.lazado@nofima.no 🖃 Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, 1430 Ås, Norway © 2020 The Author(s). Published with license by Taylor & Francis Group, LLC.

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academics, and representatives from the industry to discuss the state-of-the-art of mucosal health research and concomitantly identify gaps and issues in our current knowledge, thereby directing future development in aquaculture. The meeting was also envisioned to be an avenue to stimulate new research frontiers and opportunities, thereby cementing the relevance of mucosal health in promoting more sustainable farming practices.

The meeting gathered almost one-hundred participants (Figure 1) from around the globe (17 countries represented) and received 55 papers, 40 of which were for oral presentations while the remaining 15 were poster presentations. The talks were divided into four scientific sessions: Session 1, Mucosal structures and functions; Session 2, Mucosal health and nutrition; Session 3, Mucosal health and microbiome; and Session 4, Mucosal health and the changing environment. Each session included a plenary talk delivered by an identified authority in the field.

3. Highlights

Session 1 presented ground-breaking studies concerning fundamental questions on the structures

and mechanisms of the fish mucosa. Professor Eric Peatman from Auburn University (USA) delivered the plenary lecture, where he discussed the utilization of 'omics technology and host-pathogen models for the study of teleost mucosal structure and function'. One of the highlights from Prof. Peatman's talk was on how transcriptome analysis shed light into the molecular actors controlling the immune responses in the gills of catfish to Flavobacterium columnare. His group identified the role of rhamnose-binding lectin as a potential signature of pathogen attachment for columnaris. The talk further elucidated the role of genetics and nutrition in gill mucosal immunity. There were 12 papers presented in this session discussing topics on immune cells, mucus biochemistry, wound healing mechanism, adaptive mucosal immunity, gut immunity, and immunological rhythms. Pittman et al. presented the mucosal mapping technique that combines design-based stereology and machine learning to study the behavior of mucous cells. She emphasized that mucous cells have specific repeatable response patterns to eg. stress, water quality and feed ingredients and their morphometries have clinical importance. Nordgaård et al. presented the steric, interactive,



Figure 1. Participants of MHA2019, representing various sectors of aquaculture, including R&D institutes, academia, fish producers, feed companies, pharmaceutical companies, technology providers, and fish health service. The photo was taken at Holmenkollen on the way to the symposium dinner at Oslo Winter Park. Photo: Joe Urrutia. ©Nofima.

and dynamic components of skin mucus of yolksac salmon fry and how these features influence nanoparticle mobility through the cutaneous barrier. Mucin, a key molecule in mucus, was discussed in the presentation of Benkstander et al. where it was revealed that in salmon, the gill mucin glycans were larger, more complex and exhibited a larger range of structures than skin mucins. In addition, the talk revealed that patterns of mucin glycosylation varied markedly between healthy and infected gills. Sundh et al., Sveen et al., and Albaladejo-Riad et al. presented various aspects of wound healing in the skin of fish. In particular, Sveen et al. emphasized that the wound healing method of choice should reflect the aim of the study - focus on wound healing response or focus on factors that may enhance (e.g., diet, antibacterial agents) or impair healing (e.g., rearing environment, infections). Two papers from the laboratory of Carolina Tafalla (CISA-INIA, Spain) presented outstanding discoveries in the phenotypes and functions of mucosal B cells in teleost. Martín-Martín et al. discussed the mechanisms of how IgM+ B cells preferentially responded to thymus independent antigens when administered anally in the absence of adjuvants, while Perdiguero et al. demonstrated that, in rainbow trout, IgD+IgM- B cells are a major B cell subset among non-IgT B cells in the intestine, as they occur in the gills and IgD is clonally expanded in the gills and intestine but not in spleen. Picchietti et al. presented some recent findings on the intestinal immunity of sea bass - a marine fish where the lymphocyte distribution in digestive tract in fish was first described. The session ended with a talk (Lazado et al.) on the interplay of circadian rhythm and immunity, that despite utmost relevance (especially in infection and immunostimulation), remains a barely explored topic in fish immunology.

Professor Åshild Krogdahl, a Professor of Animal Nutrition at the Norwegian University of Life Sciences, delivered the plenary lecture for Session 2. Her talked focused on how optimal nutrition and feeding plays a crucial role in the function of the intestinal mucosa. The first part of the talk presented how common unhealthy gut is in farmed Norwegian salmon and how this affects growth, feed efficiency, and disease resistance. Some of the gut health issues include lipid malabsorption, gut inflammation, cancer, and parasites. Though it is known that a well-balanced diet is required for optimal gut mucosal function and health, the nutrient requirement in relation to mucosal health is unknown for many nutrients. It was also emphasized in the talk that mucosal health has not been a criterium for estimation of the requirement for any nutrient in aquaculture and that this must be addressed in future research. There were eight papers presented in this session, mainly focusing on dietary additives, protein replacement, and lipids, and their impacts on mucosal barrier functions. Two of the papers (Ashouri et al. and Zhichu et al.) discussed how probiotics impact mucosal health. In particular, the paper of Zhichu et al. was one of the first studies that reported how dietary probiotics could facilitate skin mucosal barrier regeneration in fish. On the other hand, Montero et al. and Torrecillas et al. presented data on the mechanisms behind the immunomodulatory action of prebiotics at the mucosa and how this feature played a role in boosting the resistance to infection pressure. Bou et al. presented a comprehensive study on the importance of omega-3 long-chain polyunsaturated fatty acids on the function and integrity of mucosal organs in salmon, wherein fish fed low levels of n-3 LC-PUFA had a high incidence of vacuolization of the supranuclear cytoplasm of the enterocytes in the mid intestine. Two papers discussed the role of nutrition on combating ectoparasitic infection, especially targeting mucosal organs. Bogevik et al. discussed how mineral ingredients might reduce the number of salmon lice in salmon, though the rearing environment might mask this effect and further suggested that this should be taken into account when planning salmon lice challenges, and also during farming operations where suboptimal rearing conditions might mask the effect of a functional feed.

Session 3 discussed some of the recent findings in microbiota research in fish, particularly their relevance in maintaining a biological barrier at the mucosa. Dr Johannes Hov from Oslo University Hospital delivered an insightful plenary lecture on host-microbiome interactions in chronic inflammatory diseases in humans, providing fish researchers with a perspective of microbiota research in a more established model. In his lecture, he pointed out that microbiota research in humans is complicated by interindividual variation and complexity of data, the influence of many confounders and very shallow understanding of the physiology. These challenges are likewise present in fish microbiota research. He further stated that microbiota research is driven by advances in big data omics and bioinformatics and the use of gnotobiotic animal models. Moreover, he presented the concept of pharmacomicrobiomics, which might pave the way in targeted gut microbiome treatment. The session included eight lectures with topics ranging from a fundamental description of mucosal microbiota to the dynamics of the microbiota in response to feed, environment, and disease. Also, several papers presented the application of gnotobiotic models and advanced sequencing platforms in characterizing fish microbiota. Legrand et al. presented the mucosal microbiota of an emerging aquaculture species in Australia, the kingfish, under different health statuses. He discussed that gut enteritis seemed to influence both the microbiota of gut and skin mucosal surfaces. Kortner et al. introduced the GutBiom Project, which aims to identify quantifiable microbiota-related markers as indicators of gut health and intestinal function for the development of functional feeds for salmon. His team identified elevated systemic short-chain fatty acid levels in salmon with signs of intestinal inflammation and thus regarded these as candidate markers of gut systemic health status. Furthermore, associations between certain dominating gut bacterial taxa, gut health, and systemic propionic acid levels were documented. Rud et al. and Klementsen et al. presented challenges and approaches in microbiota research in fish. In particular, Rud et al. presented some issues in microbiome research in fish and noted that feed microbiome could interfere with the biological interpretation of intestinal microbiome data from a feeding trial. She suggested that feed/water, mucus and fecal microbiota must be included as controls, to increase the resolution of the analysis.

The last session focused on how the changing environment, both natural and captive, could impact the mucosal health of fish. Professor Kristina Snuttan Sundell, a Professor from the

University of Gothenburg (Sweden), delivered a plenary talk on the importance of primary barriers to fish health and welfare in novel aquaculture environments. The first part of her talk revisited some key concepts of fish health and welfare, especially the interaction with stress and allostatic load. She continued her discussion by addressing the assessment of health, where she then introduced primary barrier integrity and epithelial transport as key physiological indicators of mucosal health. She presented several highlights of her research, especially on how the Ussing chamber methodology had allowed her laboratory to investigate intestinal physiology, not just molecules or genes but the live organ, and how it is affected by stressors and diseases relevant to aquaculture. The later part of her presentation discussed the dramatic developments in aquaculture production technologies (i.e., recirculation system, closed-containment system) that might significantly affect mucosal health, since these systems need higher densities of fish to achieve profitable productivity. There were twelve papers in this session, discussing topics on the interaction of stressors and mucosal health, emerging tools to study mucosal barrier functions, new treatments and production regimes, and climate change in aquaculture. Barrier functions in relation to new productions systems and regimes were the focus of two papers: Karlsen et al. discussed how salmon skin responded to different rearing systems following seawater transfer, while Cabillon et al. made an argument about the importance of mucosal health as an indicator other than growth in evaluating the benefits of exercise in fish. Four papers presented classical and emerging techniques in the study of mucosal physiology and immunology - Ivanova et al. presented the application of targeted and untargeted metabolomics to study the alterations in the metabolome of Atlantic salmon mucus induced by anesthetics; Haugen et al. presented the application of dynamic headspace gas chromatography-mass spectrometry in determining volatile organic compounds in the skin mucus of salmon with different parasitic load; Böcker et al. provided a proof-of-concept on the use of Fourier transform infrared (FTIR) spectroscopy in studying protein and carbohydrate composition of skin mucus, which has potential in assessing the

responses of fish to crowding stress and antibiotics treatment; and Fischer et al. discussed the biophysical properties of mucus through advanced techniques in rheological science. Peracetic acid (PAA) is an emerging disinfectant in aquaculture and is believed to be a highly potent oxidant with little environmental risk. Haddeland et al. discussed how a mucosal mapping strategy of the gills might provide insight into the adaptive strategies of the mucosa to repeated exposure to PAA, whereas Breiland et al. presented the comprehensive mucosal responses (i.e., morphometrics, transcriptomics, proteomics, and metabolomics) of salmon to PAA, identifying signatures related to oxidative stress. Global transcriptomic responses at the mucosa allowed Zindrili et al. to identify molecular responses to varying levels of Caligus rogercressevi infection in salmon, while Gjessing et al. presented the mechanisms of immunosuppression during a natural salmon gill poxvirus outbreak. The session was closed by Ytteborg et al. with some results from the EU H2020 Project Climefish. She presented some predicted scenarios of a changing environment, particularly related to thermal tolerance, acidification, reduced oxygen, and diseases and parasites. Moreover, she discussed that even the mildest IPCC climate scenario would impact fish mucosal health (i.e., salmon); thus, long-term studies are needed.

There were fifteen posters presented at MHA2019, including topics on innate and adaptive immunity at the mucosa, wound-healing mechanisms, mucosal responses in ecotoxicological research, and mucosal vaccination.

4. Mucosal health – a timely, relevant, and important field in aquaculture research

The 3-day symposium undoubtedly cemented the importance of mucosal health in modern aquaculture. There are five important ideas that were highlighted in the presentations and discussions during the meeting. *First*, fundamental research plays a key role in the future of mucosal health research in fish. Despite the significant strides made in unraveling the features and peculiarities of mucosal immunity in teleosts,

there remains a myriad of questions left unanswered. In particular, we have a partial understanding of the organization of the mucosal lymphoid system in fish, but there remains a significant number of questions concerning the mechanisms of adaptive immunity at the mucosa. Having answers to fundamental questions about how the mucosa is organized, functions, and adapts, might facilitate strategies to perform targeted prophylaxis and therapies. Second, modern technological platforms will not make classical techniques obsolete, but rather facilitates a more in-depth and multidisciplinary investigations of the mucosa. Both methodologies have advantages and disadvantages and researchers are encouraged to apply both to scrutinize the features and responses of the mucosa. Third, human and fish microbiota studies share a lot in common, particularly in terms of challenges in how to best extract information from sequence data. Both models have shown that a healthy and wellfunctioning mucosal microbiota significantly influences the overall health status of the host. The concept of targeting the microbiota for therapies is currently being explored in humans. Though this might have technical difficulties in fish models because of the water matrix that poses a potential confounding factor, gnotobiotic systems and nanotechnology have the potential to address the issues. Fourth, mucosal health research is no longer a topic confined within fish immunologists. Though most of the studies are still focused on the role of mucosal barriers in defense, advances in recent years have identified other aspects, specifically their biophysical characteristics. Besides immunologists, there are already aquaculture engineers, nutritionists, chemists, and computer scientists, amongst many others, who are on board in advancing this field in aquaculture. Fifth and last, studies presented at MHA2019 unequivocally substantiated that mucosal barriers are sensitive to external stimuli, including stressors, pathogens, suboptimal rearing conditions, and new production systems and regimes, to name a few. This offers possibilities to exploit the sensitivity of the

mucosa to develop a suite of tools that aim to assess fish health, and hopefully, be adapted in farms as an early warning system. This will, in turn, shift the paradigm of fish health management from reactive to proactive, to hopefully, predictive.

The next meeting is planned for 2021, in Spain.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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ORCID

Carlo C. Lazado D http://orcid.org/0000-0002-2823-2669

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