Heart of the matter

Chris Mitchell reports on the key messages from the 2022 TriNation meeting in Edinburgh

The TriNation initiative brings together fish health experts from Norway, Scotland and Ireland to share information and insights into cardiomyopathies affecting farmed salmonids, largely in these territories. The most recent meeting took place in Edinburgh last November.

If any of the 23 presentations given at the 2022 TriNation meeting struck a chord with the illustrious figures adorning the hall of the Royal College of Physicians in which the meeting was held, it might have been those addressing heart health!

With heart health now deemed a significant contributing factor to mortality, delegates were informed that fish suffering from cardiomyopathy syndrome (CMS) fare worse if their heart morphology is abnormal. Abnormal morphology can include rounded ventricles, crooked bulbus arteriosus and a thicker compactum. The latter dysmorphia can be more prevalent in S0 smolts than S1 fish, which have developed more slowly. Heart health, it would appear, can also be influenced by temperature. Acute thermal shock can result in a reduced arterial blood (and by extension oxygen) supply to the myocardium.

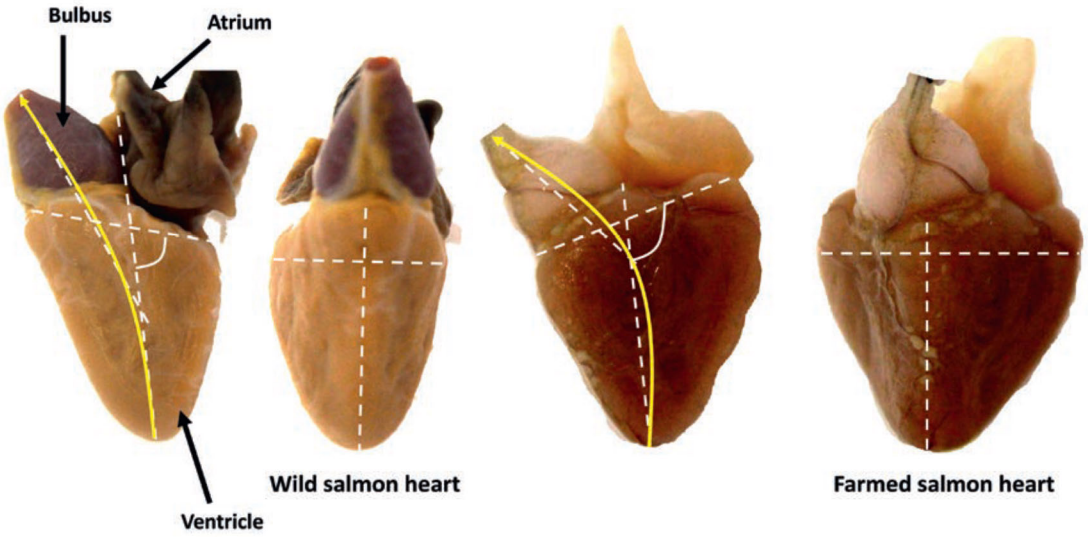
Recently, hitherto unexplained lesions have been described in this area and are being attributed to coronary artery spasm, which is well described in human medicine. Thermal shock can come either in the form of both rapid uplift in temperature, such as that used in delousing, or when fish are transferred from one site to another. Indeed, ventricular lesions have been observed in rainbow trout transferred to seawater at 2–4°C.

The impact of temperature on trout heart health per se is significant. Fish, when injected with PRV-3 and held at 5°C, exhibited higher levels of heart pathology than similarly treated fish at higher temperatures. This has been attributed both to higher (PRV-3) viral replication, combined with a reduced rate of viral clearance by the host at lower temperatures in the described study.

The ability of the host to respond to piscine myocarditis virus (PMCV) can vary, it seems, throughout the course of infection. A variety of responses, including the regulation of interferon, proteolytic enzymes as well as antigen processing and presentation, occur at different times and can be mapped over the course of an infection, and this has been reported in the hearts of PMCV-infected fish. Significantly, several immune response-related pathways, in the study described, were down regulated at eight weeks post infection and this may be a critical time point in the development of the disease, particularly in the heart.

It has been postulated that PMCV, in line with other members of the Totiviridae family of viruses to which it belongs, may require single-celled organisms within salmon to successfully replicate rather than host cells. The dominant form of viral RNA present in the spongiosum of the heart is single-stranded (messenger) RNA with little or no evidence of the double-stranded version. The suggestion is that without the framework necessary for the assembly of the outer capsid of the virus, it cannot complete its life cycle in the organ in which it is principally found.

In addition to immune regulation as a response to infection, there are a variety of other responses that can be measured and monitored



> The development and adoption of new diagnostic techniques will be vital

Opposite: Participants at TriNation 2022, Royal College of Physicians, Edinburgh
Above: The hearts of wild Atlantic salmon (left) are differentiated from those of farmed ones (right) by having a sharp triangular-shaped ventricle in contrast to a rounded one. From "Heart Morphology in Wild and Farmed Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss*" in *Diseases of Aquatic Organisms,* January 2004. Johansen R". Illustration by Ida Beitnes, NMBU, Norway

in the blood of infected fish. A field-based proteomic study of salmon undergoing challenge with CMS demonstrated that, in addition to four biomarkers traditionally associated with cardiac disease (creatine kinase, lactate dehydrogenase, salmon cardiac peptide and troponins), over 100 other proteins were observed to be elevated in fish that were infected with PMCV, but not clinical for the disease.

Establishing the identity of putative biomarkers for particular disease conditions in fish is currently being investigated for a number of species. It is hoped that either individually or as part of a panel, biomarker profiles can be useful as predictive tools in the early diagnosis of disease as well as other health issues for which the use of artificial intelligence and the mining of large data sets will be crucial.

The development and adoption of new diagnostic techniques will be vital to keep pace with the developments and changes in production methods such as recirculating aquaculture systems (RAS). Techniques such as microfluidic qPCR are being developed to accelerate and expand capability in areas where multiple microorganisms may be present in an attempt to distinguish the problematic from the benign.

As important, particularly in RAS aquaculture, is an improved understanding of how cohabiting fish represent a threat to each other from the perspective of pathogen shedding. The infection dynamics of pre-smolts infected with PRV-1 has shown that whilst growth of infectees is significantly compromised when compared with control fish, the former do not appear to shed enough virus to threaten the latter during the study period (between 10 and 31 weeks post infection). Thus, whilst this virus can be persistent in the blood and lymphoid organs, shedding may often be at levels too low to establish horizontal transmission.

Viral diseases impacting the heart, for which the most serious in salmon is arguably CMS, was mooted, along with sea lice, as the most important problem facing Norwegian aquaculture, accentuating the need for diagnostic methods to be improved. These include the detection of PMCV-specific RNA in blood plasma as an early indicator of viraemia, which was suggested as being a more sensitive and robust method of detection than immunohistochemistry whilst still a valuable support to traditional histopathology.

The continuing importance of histopathology as the gold standard differentiator of the three main viral induced myocarditis-related diseases – CMS, pancreas disease (PD) and heart and skeletal muscle inflammation – was stressed. In assessing cardiac health in this way, the development of a universally accepted method of scoring is essential. Currently there isn't one, leaving a need for such a documented and collaborative process to be developed.

Molecular studies are vital both for the development of vaccines (particular against pathogens that are difficult to culture) and improving our understanding of how pathogens change as they infect and pass through populations. Variations in viral genomes are known to influence the host-pathogen relationship in many mammalian viruses. For PMCV, different rates of infection progression have been observed but not yet explained. Current work is underway to examine how defective, or altered, viral genomes in both PCMV as well as salmonid alphavirus (SAV) 2 and 3 may influence both disease transmission and progression, as well as the development of effective vaccines.

Our ability to rapidly develop nucleic acid vaccines was no better illustrated that in the case of SARS Covid-19. Vaccines for viral diseases of fish are now also the subject of intense research, with both plasmid and mRNA versions under investigation. Vaccine effectiveness not only comes in the form of preventing mortality, but also in reducing viral shedding as well as internal tissue damage within infected fish. Additionally, a comprehensive programme of vaccination has been identified as a key component in keeping the Nord-Trøndelag region of Norway free of SAV 2, which historically had been challenging in this region.

In the early days of TriNation meetings, discussion about vaccines was focused on a single product. Much has changed since then and now both the development of new vaccines, and efficacy of existing ones, enjoys its own programme session. Accordingly, new trial data was presented on the comparative efficacy of a nucleic acid vaccine to PD and novel research into the development of a nucleic acid vaccine to CMS was presented.

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The (formerly PD) TriNation initiative was established in 2005 to integrate and focus the activities of academia and industry from Norway, Ireland, Scotland and more recently other salmon farming regions. The next meeting will be in Norway in spring 2024. For details on authorship of the material summarised here, readers are encouraged to visit www.trination.org