Better together – so it seems!

Any concerns that interest in pancreas disease (PD) and related conditions had waned since the last Trinations gathering in Leith in 2012 were soon dispelled as registrations for the 13th meeting in February of this year exceeded available spaces. And so it was to a packed house in an unseasonably dry Trondheim that Susie Mitchell got things off to a very informative start with an update on the PD situation in Ireland over the past year.

There have been 6 years since 2002 where average mortalities attributed to PD have exceeded 10% and, alarmingly, 2013 was one of them at 13%. This was particularly disappointing given the previous two years of low mortalities at 4%. One explanation might lie in the persistence of Amoebic Gill Disease (AGD) as Ireland's number one fish health issue in aquaculture. Many sites have concurrent infections of PD and AGD. In these cases, there is a clear indication that gill recovery following treatments is retarded, and re-infestation hastened, when compared to sites where AGD presents on its own.

Replacing her epidemiologist hat with an Industry one, Susie went on to report observations she had received from the farmers themselves. These included a suggestion that vaccine efficacy had not been as robust as in previous years although, in terms of mortality, vaccinated stocks still enjoyed a 2% advantage over non-vaccinates. Genetic provenance was also cited as a predictor of observed susceptibility to PD with some strains of fish faring better under challenge than others on the same site. Of perhaps more significance was the corroborating observation that concurrent infections of PD with AGD are synergistic both in the way they protract clinical disease as well as depreciate the effectiveness of freshwater as a method of treating AGD. In fact, avoiding bath treatments is regarded as a way to mitigate the impact of clinical PD!

Whilst winter Olympians in Sochi were focussing on *getting in the zone*, Anne-Berit Olsen, a regular contributor to the Trinations gathering informed us that if you are a salmon wanting to perform well in Norway, you're probably better-off out of the zone! Actually, there are two zones to be avoided, each identified by the strain of Salmon Alpha Virus (SAV) which resides there. Between 1996 and 2010 SAV3 enjoyed exclusivity as the increasingly abundant perpetrator of debilitating and deadly PD amongst farmed salmon in the west of Norway. Then in 2010 along came SAV2 into Nordmøre and Søre Trøndelag where, over the last two years, it has spread with alarming pace. Interestingly though the success of SAV2 has been concomitant with a welcome reduction in cases of SAV3 by nearly 50% since 2012. Much of this reduction appears to have been in the region of the Hustadvika firebreak in Møre og Romsdal, indicating that containment appears to be working! Of course, neither strain is desirable, but it does appear that SAV2 is generally associated with lower mortalities than its southern cousin, but not in concurrent (non-SAV) infections. Both strains, however, are associated with negative impacts on carcass quality.

As efforts to limit and even reverse the spread of SAV3 appear to be yielding results, focus will now need to be directed to SAV2 to stop it extending further north and south.

Whilst clarity and demarcation characterise the SAV story in Norway it is the often insipid nature of SAV infections in Scotland that tends to make them less of a headliner in this corner of the salmon farming world. Sara Pflaum of the Fish Vet Group reported that between 2012 and 2013 just over 8% of Scottish sea sites reported clinical disease from SAV infection. Other related conditions, collectively termed in her title 'viral myopathies' (VMPs), include Piscine Orthoreovirus (PRV) which is fairly ubiquitous but of uncertain clinical significance and Piscine Myocarditis Virus (PMCV) which is rather less common but is the established cause of Cardiomyopathy Syndrome, a condition largely confined to the Northern Isles.

Moving from Scottish matters to Chilean ones, the conference appreciated an update from Sernapesca on the VMP situation in that part of the world. Continued surveillance and detailed sampling has so far revealed no evidence of SAV or PMCV, however Marcela Lara Fica did suggest that PRV had been detected by PCR in the X, XI and XII regions although it had not been associated with clinical disease or indeed pathology; so shedding further darkness on the relationship between this virus and tissue myopathy.

This would appear to endorse David Cockerill's preference for focussing on pathology when it comes to PRV rather than simply PCR. In his presentation as an Industry health practitioner, Dave drew attention to the negative impact which myopathy has on carcass quality. In fact, plots of Creatine Kinase (CPK) levels in farmed stock correlate well with subsequent levels of customer complaints about damaged carcasses; not too surprising for an enzyme which is used as a marker for muscle damage. Interestingly though is that whilst the presence of SAV has diminished on the farms under Dave's care over the past 5 years (and incidences of CMS are now considered rare) the finger is beginning to be pointed at PRV which is now considered fairly ubiquitous on Scottish sites. More histopathology combined with more targeted PCR might begin to unravel the relationship between this virus and costly muscle damage.

In addition to PCR the use of biomarkers may play a role in allowing farmers to assess skeletal muscle myopathy without the need for lethally sampling fish. In his description of a project based at Glasgow University and supported by Biomar and Marine Harvest, Mark Braceland reported that whilst non-specificity has ruled out both Aspartate and Alanine transaminases as unique biomarkers in this role, surveillance of Enolase 3, a muscle specific biomarker, may prove more useful both in terms of health management as well as a predictor of (white) muscle quality.

Muscle lesions and subsequent fillet downgrades were also cited as a cause for concern by Dave Cockerill's fellow Marine Harvester, Solveig Gaasø who addressed the conference about her experiences of farming in the SAV 2 zone in Mid Norway. In an area where both SAV2 and PRV are abundant as co-infections, teasing out the impact on carcass quality which each virus may have, jointly or severally, is poorly understood.

Co-infection between SAV 2 and PRV is also the experience of SALMAR on their sites in both Trøndelag counties as well as the district of Nordmøre. Whilst Arne Guttvik indicated that SAV 2 was characterised by low mortalities, reduced growth is the main problem.

But what of the impact of PRV infection on its own? A collaborative investigation partnered by Marine Harvest, Nofima, Patogen and Novartis as well as the Veterinary Institute and NMBU has

been trying to reveal the significance of this Orthoreovirus in fish by increasing our understanding of its biology and host response.

Perhaps of significance is the fact that PRV can enter, reside and replicate in fish erythrocytes which it does in up to 50% of an infected host's red blood cell population quite soon after initial infection. This has been shown to happen prior to the virus entering the ventricle of the heart where it has been associated with pathology consistent with HSMI (heart and skeletal muscle inflammation). Interestingly it has been shown that fish erythrocytes, so infected, are capable of mounting an anti-viral response initially through the production of interferon which is the precursor to their own destruction (as virally infected cells) through a cell mediated immune reaction propagated in the spleen.

Of course, when any syndrome without a cause meets a cause without a syndrome, theories start to fly. However, correlation between the presence of this virus and HSMI and also it seems, Haemorrhagic Smolts Syndrome, must be viewed with interest. For the farmer and fish processor too, any virus with a predilection for blood cells must be viewed with extreme caution. I am sure we will hear more from Maria Dale and her colleagues at future Trination gatherings.

Good old fashioned sound management appears to have helped Lerøy significantly improve their ability to mitigate the occurrence and impact of pancreas disease in their operations. Synchronised fallowing, a move towards fewer but larger sites as well as careful attention to well boat management have all, according to Bjarne Reinhert, played a role in reducing the impact of this disease in the PD heartland that is Western Norway.

Well boat management, or actually a lack of it, was just one theme in a refreshingly candid exposition on the potential ease with which infectious aquatic diseases can be spread across quite considerable distances. Karl Frederik Ottem, who took the stand on behalf of Cermaq Norway, clearly believes that more attention to detail in the management of smolt transfers from the south of Norway to their operations in Finnmark may have prevented the introduction and subsequent spread of SAV 3 to this part of the world. Well boat hygiene, route management as well as smolt health and quality were all cited as contributing factors to this unfortunate transmission. Karl was roundly commended for his frank and open presentation which reflected well the principles of the Trinations ethos.

It is precisely the measures outlined by Karl which, not surprisingly, featured in Stian Johnson's summary of how the Norwegian Food Safety Authority handles SAV in areas where it is a problem. In each zone, demarcated by each strain of SAV (3 or 2) and delineated by the Hustadvika 'firebreak', a set of sanitary measures has been introduced to control and diminish both the prevalence and incidence of conditions attributed to the two strains. In addition to this, eradication of each strain north of their current extent remains a key objective. To achieve this tight controls on fish movements, transport protocols, as well as well boat and net hygiene have been imposed. Virus surveillance and synchronised fallowing also contribute to both understanding and hopefully reducing the spread of the two viruses. Although the Authority normally allows the harvesting out of populations which present with clinical signs of disease, they nevertheless reserve the power to enforce slaughter.

Whilst the movement of stocks is quite easily regulated, the movement of water is not; a point which concerns Anne Stene of Aalesund University who has discovered that each of the viruses of interest to the Trinations gathering can be found in the lipids which egress from dead salmon so infected. She has also confirmed that SAV 3 isolated from this material is infective. This being the case, Anne has raised the question of whether lipids derived from dead salmon may, by virtue of their tendency to float, provide the virus with an easy ride on prevailing currents thus raising the spectre of long-distance transmission! Clearly an area which warrants more investigation and possibly not just with SAVs!

Of course, the transmission of either virus is undesirable but Mona Dverdal Jansen of the Veterinary Institute in Oslo has attempted to compare the outcomes that an individual site might expect depending on the strain of SAV plaguing it; is there a lesser of two SAV evils? Where clinical pancreas disease is reported it appears that the levels of mortality as well as the duration of clinical signs are lower when SAV 2 is involved than with SAV 3.

Mona's colleague and frequent contributor to the Trinations conference, Torunn Taksdal continued the comparative theme but from within the aquarium rather than the ocean. By setting up challenges under controlled conditions Torunn and her team were able to demonstrate that whilst both SAV 2 and 3 are readily transmissible through the water column under an experimental infection regime, the mortalities associated with SAV 3 infection were significantly higher than those arising from SAV 2.

Inter-strain variation in pathogenicity such as that described by Torunn shows how one class of quite similar viruses can nevertheless vary biologically. Petter Frost from MSD pointed out that whilst molecular geneticists have been able to tease apart phylogenetic sub-types within the six basic strains of SAV, this is often achieved by identifying tiny variations on the genome. This is of course useful where forensic differentiation is required, say for the purpose of tracking origins of infection or geographic clustering. However genomic variations may not always be consistent indicators of biological ones a point which Petter was keen to illustrate in his presentation on antibody cross-neutralization studies across the six strains of SAV. This serological approach revealed significant variation in the extent to which antibodies raised against one strain of SAV could neutralize virus from each of the others. Importantly though, cross neutralization could be demonstrated across the spectrum of SAV strains although less convincingly with SAV 6. Petter went on to suggest that, if serological criteria were still used to subtype viruses (as was historically the case), then SAVs would be regarded as a single serotype!

Whilst antibodies bind to viruses with varying degrees of affinity so too it seems do viruses enter and replicate in tissues – so called 'tissue tropism'. Work in this field by Tharangani Herath and her colleagues at Stirling University was presented in her absence by Manfred Weidmann the new head of virology there. After impressing upon the audience that he is a WIDEmann rather than a WEEDmann, Manfred launched into a detailed exposition of the differences in the effect of SAV 1 in gill tissue compared to heart tissue (both of course subject to viral exposure in farm infections). It seems that whilst the heart tissue reacts to clear the virus through potent interferon mediated mechanisms resulting in the observed myopathy, the gill tissue does not. In fact, it is believed that in gill tissue the virus is capable of modifying the host response in a way that allows it to replicate and persist there.

Perhaps herein lies some insight into why fish with PD that are co-infected with AGD take longer to recover from the latter if perhaps the tissue under attack from amoeba is locally immunosuppressed by the virus.

SAV particles in gill tissue as revealed by PCR is currently being evaluated by AFBI in partnership with MSD as part of the latter's 'PD monitor' programme in the UK and Ireland, according to Dafydd Morris of that company. This will assist not only in mapping the virus but also in the continual development of diagnostic tools. To date, since 2008, MSD have, in Scotland alone, sold 61 million doses with a peak in sales (to date) of over 16 million in 2012.

In response to a question from Industry about any movement in the development and availability of new vaccine solutions to diseases originating from SAV infections, MSD responded that, as an animal health company, new product development was a continuous process for them. When asked the same question PHARMAQ replied that whilst they too were continuing with development, access to (and so availability within) the marketplace remained fettered.

The development of vaccines to provide protection to fish against viral diseases has been progressing for about the same length of time as fish breeders have been developing fish stocks to enhance their own immunity. But does vaccine development compete with or augment the process of breeding for disease resistance? Nina Santi of Aquagen thinks that both approaches may act synergistically. Using heart lesions as an indicator of effect, Nina, in partnership with MSD, compared the impact of vaccine with that of the IPN QTL in reducing heart pathology in SAV infected fish; the IPN QTL having been chosen as being also correlated with PD resistance. Whilst the vaccine still delivered a benefit in fish which were homozygous for *absence* of the QTL, heart lesions scores were significantly reduced in vaccinated fish which were either homozygous or heterozygous for its presence; the effect being most amplified in homozygotes.

Moderating the effects of pancreas disease through dietary intervention began over 25 years ago when the disease, correctly described as a pancreatic condition but incorrectly of nutritional aetiology, was first investigated. With the discovery of SAV as the agent responsible for PD, emphasis has shifted away from digestive support to more targeted intervention. Skretting presented data from trials of their Protec™ diet used in conjunction with, and without, PD vaccine. By monitoring plasma C-Reactive protein (CRP) as a 'precursor of mortality' the best outcome (lowest plasma CRP levels) was achieved, under aquarium conditions, when both mitigants were applied. Similar patterns of benefit were achieved (under similar conditions but without a trial of vaccine) by using the React Pan recovery diet, the most striking benefit being the reduction in mortality from 10% in controls to 1% in test fish.

EWOS have chosen to target inflammation as their focus for the dietary modulation of the economically significant myopathy diseases of salmonids. Using lower energy diets with an optimal balance of Omega 3 and 6 and which avoid immune stimulants as well as containing anti-inflammatory factors, trials have shown significant benefits through the reduction in scored tissue pathologies associated with PD, HSMI and CMS. These observations, in the case of the later two conditions, were backed up by a *depression* in the expression of genes associated with inflammation as well as an *elevation* in gene activity associated with the desaturation of fatty acids, a key component of the anti-inflammatory pathway.

The depression of inflammatory marker genes in rats which have been fed Krill oil has attracted the attention of BioMar who, in partnership with AkerBioMarine, see this resource as offering a number of benefits to salmon prior to any health insults, but also during convalescence from CMS, HSMI and winter sores. Krill oil is composed primarily of phospholipids, as opposed to triglycerides which constitute fish oil. This confers a hydrophilic property to the oil, hence its integral role in cell membrane structure. Initial trials have indicated that by including Krill oil in diets fed to salmon prior to challenge by, as well as suffering from, HSMI and CMS, the expected mortality and pathology associated with each condition can be reduced.

Perhaps if there was a single message to have come out of the Trondheim meeting it was a unifying one. In the same way that viruses appear more damaging when co-infecting, so too it seems does a multifarious approach to combating them appear more effective – *'better together!'*