International Gill Health Initiative 2017
University of Bergen, April 27-28

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At a glance – IGHI Program

Day 1 – 27 April 2017

Chair	Time	Title	Presenter
	08:30	registration open	
	08:45	registration open	
	09:00	registration open - COFFEE	
	09:15	registration open - COFFEE	
	09:30	registration open - COFFEE	
	09:45	registration open - COFFEE	
	10:00	Introduction and welcome	Mark Powell/Christine Huynh
	10:05	Welcome to the University of Bergen	Ørjan Totland
CH	10:15	An update on Gill health in Norway 2016	Anne-Gerd Gjevre
	10:25	Gill disease situation 2016-2017	Mar Marco-lopez
	10:35	Update of gill health in Scotland	Angela Ashby
	10:45	Gill Disease - an Australian perspective	Troy Hein
	10:55	Gill health update - Chile	Sonia Stolz
	11:05	MHS Gill health in Scotland 2016-2017	Martin Røed
	11:15	AGD but no treatment in 2016	Stine Kolstø
	11:25	Gill disease: What did last year look like?	Stian Nylund
	11:35	Gill health research at the University of Tasmania	Barbara Nowak
IB	11:45	**Discussion - the state of gill diseases**	
	12:15	**LUNCH**	
NR	12:45	**Epidemiological tools for studying gill disease**	**Edgar Brun**
	13:20	Pathogens causing gill diseases in Norway.	Are Nylund
	13:40	Investigation of co-infections with pathogens associated with gill disease in Atlantic salmon during an amoebic gill disease outbreak in Ireland	Jamie Downes
	14:00	Longitudinal study of putative pathogens of Atlantic salmon (Salmo salar L.) complex gill disease	Ana Herrero
	14:20	Gill disease in Atlantic salmon - studies of multiple factors in challenge models	Anne-Gerd Gjevre
	14:40	**COFFEE**	
AA	15:00	Clinical approach of the main pathological manifestations present in Chile that affect the gill health in salmonids .	Alejandro Heisinger
	15:20	Salmonid Gill Poxvirus – hallmarks of typical infection and disease	Ole Bendik Dale
	15:40	Epitheliocystis - usually benign but sometimes lethal	Barbara Nowak
MML & AGG	16:00	**Discussion - case definitions and mulifactoral gill disease syndromes**	
	17:00	End day 1	

Day 2 – 28 April 2017

Chair	Time	Title	Presenter
	08:00	welcome to day 2	Mark Powell
MC	08:10	Non-lethal molecular diagnostic test for Paramoeba perurans - experimental and field data from Norway	Hege Hellberg
	08:30	Non-Lethal skin and gill biopsies for Mucosal Mapping™ of Salmon Health – almost good to go!	Karin Pittman
	08:50	The gill parasite Paramoeba perurans compromises aerobic scope and swimming capacity in Atlantic salmon Salmo salar	Malthe Hvas
	09:10	Hypoxia tolerance during amoebic gill disease in Atlantic salmon (Salmo salar)	Morten Lund
	09:30	Physiological pathogenesis of AGD	Mark Powell
	09:50	**COFFEE**	
BN	10:10	Atlantic salmon physiological and immune response to amoebic gill disease and insight into the biology of the amoeba	Ottavia Benedicenti
	10:40	New smolt analysis shows that gill health affects the smoltification process	Elise Hjelle
	11:00	Genetic parameters for resistance to AGD in Atlantic salmon	Bjarne Gjerde
	11:20	The development of autogenous vaccines against Amoebic Gill Disease in the Atlantic salmon: an update	Sophie Fridman
MC & GR	11:40	**Discussion - knowledge gaps in understanding disease**	
	12:00	**LUNCH**	
AA	12:30	Gill Health Focus at Cargill	Ragna Heggebø
	12:50	A comparison of in vitro and in vivo results of potential functional feed candidates	Sindre Rosenlund
	13:10	Development of a functional diet against Amoebic Gill Disease	Julia Mullins
	13:30	Importance of nutrition on gill health and diseases	Rune Waagbø
	13:40	Snorkel cage barrier cage technology use and AGD infection	Lena Geitung
	14:00	Scottish research priorities for gill health management	Robin Shields
	14:20	**COFFEE**	
IB & GR	14:40	**Summing up - outcomes and the way forward**	
	15:00	End	

KEYNOTE SPEAKER

Epidemiological tools for studying gill disease

Edgar Brun

Dr Edgar Brun is an epidemiologist with the Norwegian Veterinary Institute with many years of experience in fish health related epidemiological projects including heart related diseases, and pancreas disease. Edgar has published widely on the topic of epidemiology and the associated tools for studying and evaluating diseases in farmed fish. The application of these tools and approaches to gill health related issues will be the focus of discussion.

Abstracts

UPDATES FROM AROUND THE GLOBE

An Update on Gill Health in Norway

Anne-Gerd Gjevre

Norwegian Veterinary Institute

In 2016 Norway produced about 1.2 mill tons of Atlantic salmon, 84 500 tons of rainbow trout and 25 mill cleaner fish (mainly lumpfish). Gill diseases are not notifiable in Norway. Hence, the fish health services are best updated on the situation. This update is mainly based on a survey conducted by the NVI in connection with data collection for the annual Fish Health Report. 37 persons working in 19 fish health services and 10 inspectors in the Norwegian Food Safety Authority participated in the survey. Additional information was collected from private laboratories. Generally, gill diseases have had great economic impact in the Norwegian salmon industry for many years, and the situation has not improved in 2016.

AGD has certainly come to stay. AGD is detected both in salmon and cleaner fish. The situation in 2016 was much like in 2015, i.e. did not become the severe threat we feared in 2014. This might be due to: 1) the industry is more experienced in handling the disease and seems to control AGD by gill scoring and early treatment with H_2O_2 or fresh water; 2) the summer in 2015 and 2016 had more rainfall and the sea temperatures were lower compared to previous years. In 2016 *Paramoeba perurans* was detected by real-time (RT)-PCR from the county Vest-Agder to Nord-Trøndelag. However, outbreaks of AGD were not detected north of Nord-Trøndelag. In September to November 2016 the number of treatments increased at sites on the southwest coast of Norway.

The complex gill diseases where several agents are involved, seems to give most concern in Norway. In 2016 the NVI **detected Salmonid Gill Pox Virus (SGPV)** in 11 smolt producing- and 9 on-growing farms with salmon. Infection with SGPV was on national basis, ranked at the same level as infection with IPNV, harmful algal blooms and jellyfish. In some smolt producing farms, however, the infection with SPGV can cause very high mortalities. *Ca.* Branchiomonas cysticola and *Desmozoon lepeophtherii* are also reported to cause problems in some smolt producing farms.

Gill health in Ireland in 2016

Mar Marcos-Lopez, Felix Scholz, Susie Mitchell, Hamish Rodger

Fish Vet Group Ireland, Unit 7b Oranmore Business Park, Oranmore, Co. Galway, Ireland

Gill health continues to be one of the main health challenges for the Atlantic salmon industry in Ireland. During 2016, all new smolt inputs (2015 S0s and 2016 S1s) became infected with *Neoparamoeba perurans* and displayed clinical amoebic gill disease (AGD) a few weeks or months after sea transfer. Overall however, mortalities and number of bath treatments (approx. 90% freshwater and 10% hydrogen peroxide) were low. An increase in proliferative gill disease and gill bleeding was observed in fish close to harvest size. A full diagnosis was not carried out in all cases, but phytoplankton species (*Ceratium* sp. and *Karenia mikimoto*) were diagnosed as the primary cause in two significant cases. Pathogens known to be associated with complex gill disease (i.e. *Desmozoon lepeophtherii*, salmon gill poxvirus and *Branchiomonas cysticola*) were also detected, but their role on the observed pathology is unclear. Clinical AGD has also been diagnosed in lumpfish and wrasse species in Ireland, and significant AGD-related mortalities occurred in lumpfish both in rearing facilities and in sea cages. The main challenges and research and management needs for both Atlantic salmon and cleaner fish in regards to gill health will be discussed during the presentation.

An Update on Gill Health in Scotland

Angela Ashby

Fish Vet Group UK

Gill health is widely considered to be one of the most significant health challenges facing the Scottish salmon industry. This presentation will provide an overview of gill health in Scotland, including clinical observations from the field and diagnostic trends.

Gill disease- Australian perspective

Troy Hein

Tassal Operations, Tasmania Australia

Gill diseases have great economic significance in Australia due to losses (direct) and the added cost of production from freshwater bathing. Amoebic gill disease (AGD) has been a major challenge for the industry, but through the selective breeding program, inroads have been made into AGD resistance/resilience of stock.

Emerging diseases that have seasonal impacts include necrotic branchitis associated with hydrozoan injury, and secondary infections with *Tenacibaculum* sp.- causing bacterial plaques on gills. These gill diseases are further compounded by thermal stress as the severity and prevalence of gill necrosis occurs in the summer months as temperatures peak (January to March).

Further research needs to be conducted in this area to understand the progression of disease caused by Australian species of hydrozoa; seasonal variation of biofouling species; and management practices that can be employed to mitigate gill injury. There also needs to be a close examination of in-situ net-cleaning practices and the role of total suspended solids and "blasting" effect of net cleaners which can dislodge and break-up hydrozoan colonies causing dispersal of nematocysts in the water column.

Gill health update – Chile

Sonia Stolz

Fish Vet Group Chile

MHS Gill health in Scotland 2016-2017

Martin Roeed

Marine Harvest Scotland

Marine Harvest is dedicated to the health and welfare of our fish, and we are passionate about solving the challenges we face in our operations. A healthy salmon is a high-performing salmon and in 2016 Marine harvest Scotland faced some challenges, related to poor gill health (AGD, PGD, algae bloom, jellyfish). Poor gill health both kills fish directly and also weakens fish that then succumb to other pathogens, it is possibly our most significant fish health challenge at present.

A complex gill disease involving bacteria, virus and parasitic organisms together can result in severe proliferative pathology with significant reduction in gill surface area. PGD has along with AGD proven to cause difficulties in operation related to handling of fish (harvest, treatments etc.)

The full impact on fish health from AGD is not fully understood; some farms can be positive, with quite high levels of amoeba for many months, but without showing any obvious negative effects, while the health of fish on other farms can deteriorate very quickly into very high mortality from low levels of infection. AGD is highly seasonal and it has a natural low presence and effect on fish health around late April/early May but by late summer almost all of our farms are re-infected and health impacted.

Spring 2017 has so far been used to battle sea lice and plan strategic treatments towards and prepare for the challenging summer months.

AGD but no treatment in 2016

Stine Kolstø

FoMAS, Haugesund, Norway

FoMAS is a fish health service company in the Southwest of Norway, and from 2013 when we had the first larger outbreak of AGD in our region we have worked closely with AGD and treatment in the field. In 2016 we had no treatment against AGD in our region, despite several diagnosis and development of gillscore. We will present our experiences from the field and compare it to previous years.

Gill disease: What did last year look like?

Stian Nylund, Pharmaq Analytiq, Bergen Norway

Gill disease has been recognized as a significant challenge during production of Atlantic salmon since the 1980s. The number of reported gill disease cases seems to increase every year and is also closely linked to high sea temperatures in late summer/early autumn. Geographically, the most strongly affected area has been, and still is, Western Norway, but in the latest years this area has slowly increased to include regions further north. Although the associated mortalities generally are low (approx. 10%), more severe isolated cases with heightened mortalities have been reported, typically following handling of fish and/or sea lice treatment.

Determining the cause of gill disease is not a straightforward task. Pathological changes in gill tissue can be caused by both environmental factors and a wide selection of pathogens. Since gill infections caused by disease agents is thought to be multifactorial they often include representatives from several different kingdoms of parasites. Common gill pathogens in Norway include intracellular bacteria (Chlamydia- or β-proteobcateria) causing epitheliocysts, exoparasites like *Paramoeba perurans* and *Ichytobodo* sp., the microsporidian *Paranucleospora theridion* and the viral agents Atlantic Salmon *ParamyxoVirus* (ASPV) and Salmonid Gill PoxVirus (SGPV). Although most of these are associated with disease in the seawater phase, several also represent a challenge in freshwater production. Determining which of these pathogens are primary- and secondary causes is a challenge, and a direct correlation with disease has only been shown for a few of them. Systematic monitoring for their presence during production using both realtime RT-PCR and histological examination can to a certain degree start to unravel the relative importance of the different agent in causing disease. The presentation will include a summary of field results and our experiences from last year's monitoring.

Amoebic Gill Disease – UTAS research update

Barbara Nowak
IMAS UTAS Tasmania Australia

Amoebic gill disease (AGD) was first reported from salmonids farmed in marine environment 30 years ago. Since then clinical AGD has been observed in fourteen countries across six continents. The causative organism, *Neoparamoeba perurans*, was described more recently. This presentation will review AGD research progress at UTAS.

We investigated the effects of fresh water treatment against Amoebic Gill Disease on the gut microbiome of farmed Atlantic salmon. The results showed high variability, in particular between different dates and potentially fish size. *N. perurans* gene sequences from isolates from different geographical locations were compared using MLST and RAPD. Further progress was made with regard to environmental detection of *N. perurans*. Challenges in the detection of *N. perurans* in benthic sediments associated with commercial salmon farming will be discussed.

GILL DISEASES IN THE ENVIRONMENT AND INTERACTIONS

Pathogens causing gill diseases in Norway.

Are Nylund

University of Bergen, Bergen, Norway

Production of Atlantic salmon *Salmo salar* in Norway has since the mid 90-ties suffered from gill diseases and the majority of the cases have been located to western Norway (Nygaard 2004). The exceptions are the parasites *Parvicapsula pseudobranchicola* that affects the pseudobranch, gills and other tissues of farmed Atlantic salmon mainly in Northern Norway (Karlsbakk et al 2002, Sterud et al 2003, Nylund et al 2005) and *Ichthyobodo* spp associated with gill diseases in both fresh and sea water in most part of Norway (Todal et al 2004, Isaksen et al 2010, 2011, 2012, Isaksen 2013). The gill diseases of Atlantic salmon in Norway have been named proliferative gill inflammation, PGI, associated with a paramyxovirus (Kvellestad et al. 2003, 2005, Fridell et al 2004, Nylund et al 2007, Steinum et al 2010), proliferative gill disease, PGD, associated with a poxvirus (Nylund et al. 2006, 2007, 2008, Gjessing et al 2015, 2017), amoebic gill disease (AGD) associated with *Paramoeba perurans* (Nylund et al 2007, 2008, Steinum et al 2008), bacterial gill disease (BGD) mainly caused by *Flavobacterium* spp. and *Tenacibaculum* sp. (Lorenzen 1999, pers.obs.), ichthyobodosis caused by *Ichthyobodo* spp (Poppe & Håstein 1982, Todal et al 2004, Nylund et al 2005, Isaksen et al 2010, 2011, 2012, Isaksen 2013), paranucleosporosis caused by *Paranucleospora theridion* (Nylund et al 2009abcd, 2010, 2011, Gunnarson et al 2016), parvicapsulosis caused by *P. pseudobranchicola* (Karlsbakk et al 2002, Sterud et al 2003, Nylund et al 2005), and epitheliocystis caused by members of Chlamydiales (*Candidatus* Piscichlamydia salmonis, *Ca.* Clavochlamydia salmonicola, and a new member of Simkaniaceae) and the β-proteobacterium *Candidatus* Branchiomonas cysticola (Nylund et al 1998, Drahgi et al 2004, Karlsen et al 2008, Steinum et al 2010, Repstad 2011, Toenshoff et al 2012, Vilinn Tolås 2012, Mitchell et al 2013, Nylund et al 2014). This presentation will give a review of microparasites involved in gill diseases in western Norway.

Investigation of co-infections with pathogens associated with gill disease in Atlantic salmon during an amoebic gill disease outbreak in Ireland

Jamie K. Downes[1,2*], Tadaishi Yatabe[3], Mar Marcos-Lopez[2,4], Hamish D. Rodger[4], Eugene MacCarthy[2], Ian O Conor[2], Evelyn Collins[1], Neil M. Ruane[1]

[1]Fish Health Unit, Marine Institute, Oranmore, County Galway, Ireland; [2]Marine and Freshwater Research Centre, Galway Mayo Institute of Technology, Dublin Road, Galway, Ireland; [3]Center for Animal Disease Modeling and Surveillance (CADMS), Dept. Medicine & Epidemiology, School Veterinary Medicine, University of California, Davis, USA; [4]Fish Vet Group, Unit 6, Oranmore business park, Oranmore, County Galway, Ireland.

On a global scale, amoebic gill disease (AGD) is the most significant gill disease affecting farmed Atlantic salmon, while a number of other agents have been identified as potential pathogens involved in gill disorders. Gill disorders can be complex and multifactorial with co-infections common on farms and there is a lack of knowledge in relation to interactions and synergistic effects of these agents. The objective of this study was to determine if and what effect a number of pathogens, namely *Neoparamoeba perurans*, *Desmozoon lepeophtherii*, *Candidatus* Branchiomonas cysticola, *Tenacibaculum maritimum*, piscine reovirus (PRV), and salmon gill pox virus (SGPV) may have on the gills during an AGD outbreak. For this, gill samples were collected from stocking until harvest, every 2-4 weeks, from a marine Atlantic salmon farm in Ireland, on which real-time PCR was used to determine the presence and sequential infection patterns of these pathogens. Finally, a number of multi-level models were fit to determine the effect of these putative pathogens and their interaction on gill health (measured as Histopathology score), while adjusting for the effect of water temperature and time since the last freshwater treatment. Results indicate that between week 12 and 16 post-seawater transfer, colonisation of the gills by all pathogens had commenced and by week 16 of production each of the pathogens had been detected. *D. lepeophtherii*, *Candidatus* Branchiomonas cysticola and piscine reovirus (PRV) were by far the most prevalent of the potential pathogens detected during this study. Once established in the population, there was very little variation in the prevalence (*D. lep* 80 to 100%, *Ca*. B. cysticola 80 to 100% and PRV 60 to 100%). Detections of *T. maritimum* were found to be significantly correlated to temperature showing distinct seasonality. While detections of salmon gill pox virus (SGPV) were highly sporadic and it was detected in the first sampling point, suggesting a carryover from freshwater stage of production. Finally, model results indicate that there is no clear interaction or synergistic effect between any of the pathogens. Additionally, the models showed that temperature, the density of *Neoparamoeba perurans* and time (weeks) since last freshwater treatment have the greatest effect on the histopathology score.

Longitudinal study of putative pathogens of Atlantic salmon (*Salmo salar* L.) complex gill disease

Ana Herrero[1*], Mark Dagleish[1], Hamish Rodger[2], Carolina Guitierrez[3], Chris Cousens[1], Jeanie Finlayson[1], Jorge del-Pozo[5], Chris Matthews[2], Giuseppe Paladini[4], James Bron[4], Alexandra Adams[4] and Kim D. Thompson[1]

[1]Moredun Research Institute, Pentlands Science Park, UK; [2]Fish Vet Group, Inverness, UK; [3]Marine Harvest, Fort William, UK; [4]Institute of Aquaculture, University of Stirling, UK; [5]Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK.

Gill disorders have become a significant problem during the marine phase of Atlantic salmon farming. The aetiology can be a single pathogen, e.g. *Paramoeba perurans* in amoebic gill disease, or associated with the presence of several agents including bacteria, viruses and parasites. Other factors, such as previous insults, environmental conditions or stress due to handling can be predisposing factors. Establishing the causative aetiological agent(s) in gill disease is frequently complicated by the simultaneous presence of various pathogens. Furthermore, the effect of interactions between these different organisms in complex gill disease is unknown. In Scotland, complex gill disease has been reported more frequently from the end of the summer until the end of winter. We performed a longitudinal study from October 2016 until February 2017 at two salmon farms in different locations on the West coast of Scotland, both with a history of previous occurrence of gill disease. The aim was to determine the correlation between gill pathology and the presence and relative levels of the putative pathogens present in the gills. Six fish were sampled every two weeks from each farm and the presence and load of *P. perurans*, *Ca.* Branchiomonas cysticola, salmon gill poxvirus and *Desmozoon lepeophtherii* in the gills and head kidney were determined by specific quantitative reverse transcriptase polymerase chain reaction (qRT- PCR). A gill scoring technique for assessing histological lesions was also undertaken in samples from these fish, the results of which were compared to the presence and load of each pathogen. The results obtained to date will be presented and discussed.

Gill disease in Atlantic salmon - studies of multiple factors in challenge models

Anne-Gerd Gjevre

Norwegian veterinary Institute, Oslo, Norway.

Gills are multifunctional organs: respiration, osmoregulation, acid-base regulation and nitrogen excretion. Both infectious and non-infectious factors are associated with gill disease in farmed Atlantic salmon in seawater. The cause of the disease is complex, and is therefore referred to as multifactorial. The presentation sums up main results form an ongoing research project. The main objective was to study the significance of and interaction between the various factors associated with gill disease in maricultured Atlantic salmon. Standardized infection models was developed. Fish were exposed to environmental factors believed to be important for the development of gill disease and simultaneously challenged with *Paramoeba perurans*.

Clinical approach of the main pathological manifestations present in Chile that affect the gill health in salmonids

Alejandro Heisinger

Multiexport Food Co, Puerto Montt, Chile

The Chilean Aquaculture production has more than 25 years, achieving a position as the second worldwide producer, its important growth as the intensification of the production has led to the presence of different diseases, where the last 5 years has seen the appearance of diseases affecting the gill health of various noxa, such as parasites, viruses, bacteria, Harmful Algal Bloom, etc.

The following paper presents the main manifestations associated to gill disease, predisposing factors, clinical diagnosis, diagnosis tools, therapeutic alternatives, as well as prevention and control measures.

Salmonid Gill Poxvirus – hallmarks of typical infection and disease

Ole Bendik Dale

Norwegian Veterinary Institute, Oslo, Norway

A brief introduction to the newly characterized virus and the emerging disease in Atlantic salmon is given. Further, our experiences regarding infection and development of typical poxviral disease is reviewed.

Epitheliocystis - usually benign but sometimes lethal

Barbara Nowak

Institute for Marine and Antarctic sciences, University of Tasmania, Launceston, Tasmania Australia.

Epitheliocystis is a gill condition caused by an intracellular bacterial infection. It has been reported from both cultured and wild fish. While different species of Chlamydia have been confirmed as causative agents of epitheliocystis in many host species, in some cases Betaproteobacteria have been confirmed as aetiological agent. Epitheliocystis is often a benign infection but can also result in a proliferative host response leading to significant fish mortalities. This presentation will review the current state of knowledge of epitheliocystis and discuss its potential significance in co-infections and other gill diseases.

DIAGNOSTICS

Non-lethal molecular diagnostic test for *Paramoeba perurans* - experimental and field data from Norway

Hege Hellberg

Fish Vet Group Norge AS

Amoebic gill disease (AGD) and other gill diseases cause large losses in salmon farming. Developing non-lethal tests to monitor fish populations for pathogens would help improve production economy and fish welfare. A non-destructive molecular diagnostic test has showed improved detection of *Paramoeba perurans* (Downes et al. 2017). Results from experimental and field testing of the method in Norwegian salmon farming will be presented. The use of the method for detection of other gill pathogens will discussed.

Refs.: Downes et. 2017 "Evaluation of non-destructive molecular diagnostics for the detection of *Neoparamoeba perurans*", Frontiers in Marine Science, March 2017; volume 4.

Non-Lethal skin and gill biopsies for Mucosal Mapping™ of Salmon Health – almost good to go!

Karin Pittman[1], Natalie Brennan[1], Steffen Blindheim[2], Linda Andersen[2] and Mark Powell[1,3]

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Mucosal Mapping is an objective, statistically robust measure of the health of mucous membranes in the barrier tissues of fish skin, gills and guts. In order to use it for non-lethal monitoring as well as analysis, we tested the applicability of various sizes of skin biopsy punches and gill clips, as well as wound healing protocols to give good fish recovery and sufficient data for analysis.

Fifty individually Pit-tagged healthy salmon (mean wt 165 g) were held in a 500 L fiberglass tank with 15 ppt water and were given a matrix combination of biopsy punch sizes (4, 5, 6, 8 and 12 mm) and 5 wound healing compounds (Epiglu, Gluture, Histoacryl, VetBond and nothing). A further 30 individually Pit-tagged salmon of similar size were kept in a separate tank, similarly anesthetized and monitored for the effects of large or small gill clips on the second gill arch. One operator did the skin biopsies and another did the gill clips. All fish were anesthetized with FinQuel and time out of water was recorded. All fish survived, except those 4 which were held out of water for 2 minutes, longer than the 1 minute which the others were subjected to. Fish were monitored for survival and welfare daily for 10 days.

All skin wounds expanded in the vertical and horizontal axes, with VetBond giving the consistently largest wound size and “nothing” giving consistently the smallest. Furthermore, some of the wound healing substances crystallized and fell off early in the post-biopsy period. The 4 mm biopsy punch was easily disrupted by the scales surrounding the intended excise spot and gave too few mucous cells for Mucosal Mapping (require >100 randomly chosen cells). The 12 mm biopsy was judged to be too large for such small fish even though all survived with no apparent infections. Follow-up samples were systematically taken to investigate for secondary fungal infections etc. The Mucosal Mapping results are being tabulated for this contribution.

The distinction between “large” and “small” gill clips was confounded by the type of scissors used, as the fragile tissue could fold around blades and need to be flicked into the histocassettes for processing. We had overlooked the effect of the grid gaps in histocassettes to release the clipped and loose gill filament tissue, and so had only a remaining 4 “large” samples available for Mucosal Mapping. These provided sufficient numbers of mucous cells on the gill filament and consistent measures of mean cell area and density. It was noticed that these healthy fish had very few and small mucous cells on their lamellae, which is the respiratory surface, and these were of a clearly smaller size and density than those on the filament or the skin. The presence or absence of these lamellar mucous cells may be the clearest candidate for an objective quantitative measure of fish health and welfare in the field.

The gill parasite *Paramoeba perurans* compromises aerobic scope and swimming capacity in Atlantic salmon *Salmo salar*

Malthe Hvas, Egil Kalsbakk, Frode Oppedal

Institute for Marine Research, Bergen Norway

The parasite *Paramoeba perurans* is an etiological agent of amoebic gill disease (AGD), a serious problem in seawater salmonid aquaculture globally. The amoeba cause patchy hyperplastic and mucoid lesions in the gills, which may become necrotic. Hence respiration may be affected, but Atlantic salmon with AGD also show increased aortic blood pressure and systemic vascular resistance. A better understanding of the pathophysiology of infected fish is warranted. In this study groups of Atlantic salmon without and with severe AGD (mean score of 4.1) were tested in a large swim tunnel respirometer in seawater at 13°C to assess swimming capacity, oxygen uptake and blood parameters. The critical swimming speed was reduced from 3.0 BL s-1 in controls to 2.5 BL s-1 in infected fish. Standard metabolic rates were similar between groups, but the maximum rate of oxygen uptake was drastically reduced in AGD fish, which resulted in a smaller aerobic scope of 260 mg O2 kg-1 h-1 compared to 410 mg O2 kg-1 h-1 in healthy fish. Furthermore AGD fish had lower haematocrit and [haemoglobin], but similar condition factor compared to controls. Before swim trials AGD fish had higher plasma osmolality and elevated plasma [Na+] and [Cl-] indicating reduced capacity to maintain ionic homeostasis, while cortisol levels were higher in AGD fish both before and after swim trials. These results show that AGD inhibits gill function, is a significant stress factor, and decreases swimming performance.

Hypoxia tolerance during amoebic gill disease in Atlantic salmon (*Salmo salar*)

Morten Lund

Norwegian Veterinary Institute, Oslo, Norway

Amoebic gill disease (AGD) is caused by *Paramoeba perurans* and causes significant gill disease in farmed Atlantic salmon (*Salmo salar*). AGD poses a serious challenge to the Atlantic salmon aquaculture in Tasmania, Norway and the British Isles and causes substantial economic losses. The histopathological changes of the gill tissue during AGD are suggested to impair important functions of the gills, i.e. the respiration and acid/base control. Furthermore, an impaired cardiac function has been suggested in AGD diseased Atlantic salmon. The hypoxia tolerance of AGD diseased Atlantic salmon may also be impaired due to a reduced respiratory surface of the diseased gills. This is important to investigate due to the hypoxic and crowding stress the fish may encounter during both AGD and salmon lice treatments.

In the present study, the hypoxia tolerance of Atlantic salmon post-smolts experimentally infected by *Paramoeba perurans* was investigated by determining the individual incipient lethal oxygen saturation (ILOS) level in a hypoxia tolerance test (HCT). Tank water temperature was 12 °C during the challenge trial. The HCT was performed 7, 23 and 36 days post infection (dpi) in a common-garden setup, including 30 – 32 individuals from the non-infected and infected groups. Macroscopic gill scoring (0 - 3) was performed on all fish included in the HCT and gill samples for RT-qPCR and histopathological analysis were collected.

The oxygen saturation level at ILOS was plotted against minutes from start of the HCT in a Kaplan Meyer plot at each time-point for each group and gill score. No difference in hypoxia tolerance between the infected and non-infected groups were detected at any time-points. There was not detected any difference in the hypoxia tolerance between the gill scores. The mean gill score in the infected group were 0.44, 1.87 and 1.9 at 7, 23, and 36 dpi, respectively. This suggests that a mean macroscopic gill score up to 2, (clinically equivalent to industry standard treatment levels) does not affect the hypoxia tolerance in AGD diseased Atlantic salmon. The low prevalence of gill score 3 (N = 7/32) at 36 dpi is not sufficient to determine a possible effect on the hypoxia tolerance at this score. RT-qPCR and histopathological analysis is pending.

Contrary to expected, the macroscopic gill score at 23 and 36 dpi remained the same in the infected group. This may indicate a difference in the ability to cause severe AGD in the amoeba isolate used in this challenge trial and is consistent with the findings in others studies where this clone has been used.

In conclusion, the hypoxia tolerance in Atlantic salmon post-smolts infected by *Paramoeba perurans* was not affected up to a mean macroscopic gill score of 1.9. A possible lower virulence in the amoeba isolate used was also observed.

Physiological pathogenesis of AGD

Mark Powell

University of Bergen, Bergen Norway

Amoebic gill disease is perhaps one of the most studied gill disease issues and an extensive knowledgebase exists on the pathophysiology and immunological responses to its manifestation. Earlier studies identified key physiological issues associated with the manisfestation of the disease in Atlantic salmon and are more recently, supported by AGD in non-salmonids such as ballan wrasse. The initial phases post-challenge with amoebae resulted in a slightly elevated ventilatory response with associated effects of acid-base metabolism (a mixed respiratory acidosis and alkalosis). Later stages of the disease development where clinical gill scores manifested, marked respiratory acidosis occurred. During the initial phases of disease, progressive increases in routine metabolic rate occurred with little measureable effect on standard or active metabolic rate (and metabolic scope). However, later stages of disease (equivalent to clinical levels where treatments are carried out), the metabolic cost of AGD was significant with large reductions in aerobic scope that was reduced in unfed (starved) fish.

Key points of relevance and impact

- Physiological responses are progressive as AGD develops
- Feeding has a significant effect on metabolic cost of AGD
- Freshwater bathing has a significant impact on the physiological responses to AGD

Atlantic salmon physiological and immune response to amoebic gill disease and insight into the biology of the amoeba.

Ottavia Benedicenti

University of Aberdeen, Aberdeen, Scotland

Amoebic gill disease (AGD) is an emerging disease in North European Atlantic salmon (Salmo salar) aquaculture caused by the amoeba *Paramoeba perurans*. Non-optimal environmental conditions such as increasing water temperature may affect AGD progression. To understand the role of predisposing environmental conditions on the biology of the parasite and on the host physiological and immune response, different clonal cultures of *P. perurans* and AGD infected Atlantic salmon were exposed, respectively, in vitro and in vivo to two different temperatures, 10°C and 15°C. Amoebae were propagated in non-axenic cultures on malt yeast agar (MYA) with an overlay of sterile seawater. Differences in growth rate among *P. perurans* clonal cultures were analysed using a negative binomial analysis in R (R software, version 3.0.1). 16S MiSeq analysis was also performed to characterise the changes in bacterial communities present in the in vitro cultures at the two different temperatures. Atlantic salmon smolts were exposed in vivo to the same temperatures used for the in vitro experiment and to an initial concentration of 500 cells/l of a clonal culture of *P. perurans* for 3 weeks. Analyses of cortisol, glucose, and lactate concentration in plasma samples, and gene expression have been performed at different time points after the challenge.

Significant differences in growth rate were detected between clonal cultures over time and the fraction of amoebae in suspension in seawater showed a higher increase over time at 10°C, whereas the fraction of attached amoebae showed a higher increase at 15°C. Cortisol, glucose, and lactate levels in plasma did not differ significantly between AGD-affected fish held at the two temperatures, while significant differences were detected in gill pathology at the two temperatures. Gene expression analysis showed significant up regulation of IL4/13 isoforms in gills at both temperatures in comparison to control fish.

The results from the temperature dependent AGD infection and in vitro *P. perurans* growth rate studies hint that increased AGD outbreaks during summer periods may not be solely due to increased thermal stress in fish but also to increased amoebae attachment at 15°C which cause an increased gill pathology.

New smolt analysis shows that gill health affects the smoltification process

Elise Hjelle, Pharmaq Analytiq, Bergen Norway

Up to 6% of the total loss of salmon and trout in the aquaculture industry is related to the freshwater phase (Tap av laksefisk i sjø, Mattilsynet 2014). Weakened smolt that survive the freshwater phase will eat less, withstand less handling and increase the risk for disease in the whole population. This adds on to an already increasing production cost. Different analytical methods are used to document the smolt status - this includes seawater challenge test, Na+/K+ ATPase test and the measurement of gene expression related to smoltification.

In PHARMAQ Analytiq we have performed Na+/K+ ATPase testing since 2001, which have generated data and knowledge from several hundred thousand fish. The need of an analysis that can generate more information than traditional methods has become more apparent with the development in the industry towards producing larger smolts, and with the increased use of seawater and salt feed in the production cycle. We have now developed a new realtime RT-PCR method called SmoltVision, to measure the seawater tolerance in salmon. In cooperation with UNI Research and the University in Bergen we have identified three genetic markers that give information on what the smoltification profile looks like at the time of sampling. SmoltVision is more sensitive to external factors that affect the fish, like water quality and gill disease, than traditional methods. The SmoltVision samples can also be reanalysed for gill pathogens if there should be any indications of such, to confirm a diagnosis. In this sense SmoltVision is also working as a welfare indicator, picking up on suboptimal conditions for the fish.

In this talk we will present field data from our new smoltification tool including how costia can affect smoltification. We will discuss the use of this tool as a welfare indicator when producing smolts.

Key points of relevance:
- New smolt analysis provide improved information during smoltification
- How Costia can affect smoltification
- SmoltVision can work as a gill welfare indicator

Genetic parameters for resistance to AGD in Atlantic salmon

Bjarne Gjerde

NOFIMA, Norway

Atlantic salmon from 100 fullsib families were tested in a challenge (Feb.-Apr. 2016) and field (Nov. 2016) test. In both tests an extended Taylor gill-score was used (0, 1, 2, 3A, 3B, 3C, 4, 5). In the field test, a gill-tissue sample was obtained by swab from the 2nd anterior gill-arch on the left side of each fish and were analyzed for *Paramoeba perurans* by qPCR; individual body weights were also recorded. In both tests the distribution in gill-score was narrow with very few or none fish with score 0, 1, 3C, 4 and 5. In the challenge test, the estimated heritability was 0.14 ± 0.06 for 1st and 0.11 ± 0.03 for 2nd gill-score, while in the field test it was 0.17 ± 0.05 for gill-score and 0.09 ± 0.04 for qPCR. The genetic correlation between the gill-scores in the challenge test and gill-score in the field test were low and not significantly different from zero, as also found in another project (RCN-235783/E40). In the field test the genetic correlation between gill-score and 1/qPCR was high positive (0.90 ± 0.16), and between body weight and gill-score high negative (-0.88 ± 0.09), while the residual correlations were much lower (-0.22 and 0.16, respectively). These results indicate that testing of Atlantic salmon for resistance to AGD should be based on gill-scores from a field test rather than from a challenge test. An estimate of the magnitude of the genetic correlation between gill-score in a field test and growth in an AGD-free test environment is required.

The development of autogenous vaccines against Amoebic Gill Disease in the Atlantic salmon: an update

Sophie Fridman[1], Isaac Vizcaino-Caston[2], James Bron[1], Giuseppe Paladini[1], David Haydon[2], Tim Wallis[2], Teresa Garzon[3], David Cockerill[3] and Alexandra Adams[1]

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Amoebic gill disease (AGD) has emerged as an increasing threat to the Atlantic salmon aquaculture industry. Originally reported in Tasmania in the mid-1980s, it has since spread to become a global problem. It currently costs the salmon farming industry millions of pounds every year in treatment costs and fish losses. Amoebic gill disease is caused by the free-living marine amoeba Paramoeba perurans, which is widely distributed in the environment, only becoming a concern when it attaches to the gills, causing lesions and respiratory distress to the fish. Unless treated, it can ultimately lead to fish morbidity and mortality. The aim of the current project, funded by Innovate UK and BBSRC and involving the University of Stirling’s Institute of Aquaculture, Ridgeway Biologicals Ltd. and Marine Harvest, is to develop and test autogenous vaccines to control AGD in farmed Atlantic salmon. To date amoebae have been collected from gills of salmon infected with AGD from Marine Harvest sites on Scotland’s west coast. The consortium is now culturing, isolating, characterising and comparing these different amoeba strains and developing vaccines based on the results. The first autogenous vaccine will shortly be efficacy tested in Atlantic salmon under experimental conditions, using a cohabitation challenge. Vaccines that prove effective will then be used to vaccinate fish in sea cages to assess vaccine efficacy in fish following natural exposure to amoeba.

Impact points:

- Amoebic gill disease (AGD) has emerged as a major threat to the Atlantic salmon aquaculture industry
- Existing control methods are expensive and logistically challenging
- Autogenous vaccines targeting AGD are under development; efficacy tests in Atlantic salmon will start shortly

NUTRITION AND TREATMENT SOLUTIONS

Gill Health Focus at Cargill

Ragna Heggebø

Cargill Innovation, Bergen, Norway

Amoebic gill disease (AGD) is a significant threat to salmonid aquaculture. The disease is prevalent in Tasmania, Australia, with an estimated cost to the industry of A$230M per year. Other salmon producing regions such as Ireland, UK and Norway are also increasingly affected by the pathogen where it is also adding significant cost and restrictions to production operations.

Dietary modulation of the parasite and the physical damage to the host are currently being assessed. Focus has been to elicit an effective immune response in the gills, manage stress associated with infection and treatments, as well as to screen a range of in-feed products with direct effect against the parasite. In this update we describe our ongoing research and collaborations to develop better tools for the industry to combat gill disorders.

A comparison of in vitro and in vivo results of potential functional feed candidates

Sindre Rosenlund

University of Bergen, Bergen, Norway

Amoebic Gill Disease (AGD) is rapidly becoming a significant health issue in Norwegian fish farming. Treatment of the disease has only two commercially available options; freshwater and hydrogen peroxide. This study aims to investigate if potential functional feed candidates can be used as a preventive measure, in stopping the disease progression. Firstly, an assay for investigating the efficacy of a feed candidate was developed. Then several potential candidates were supplied by Cargill innovation. After the screening process three candidates was picked for in-vivo direct challenge trials. The results from these trials demonstrates a strong correlation between results of in-vitro screening, and in-vivo challenge trials, based on gill scores and RT-PCR.

Development of a functional diet against Amoebic Gill Disease

Julia Mullins

Skretting ARC, Stavanger, Norway

Amoebic gill disease (AGD) caused by *Paramoeba perurans* is the leading cause of gill disease in Atlantic salmon worldwide. Despite more than 30 years of research, there are no vaccines or medicines licensed to treat AGD. There is an opportunity through diet to potentially alter both the physical and qualitative characteristics of fish mucus, and in turn influence the survival of fish affected with AGD. An in vitro plate assay was developed to screen and identify prospective compounds for inclusion in diets and to evaluate the effects of mucus collected from Atlantic salmon fed these compounds. Dietary effects of these ingredients were then assessed through fish survival and selected biomarkers in Atlantic salmon experimentally infected with *P. perurans*. Further, the same compounds were also fed to non infected fish to assess their effects upon mucus characteristics.

Experimental results showed that diet has the potential to play an important role in the management of AGD by improving fish survival, enhancing mucus defences, reducing gill damage and reducing amoebae survival.

Three key points of impact:

1. *P. perurans* survival was significantly reduced by direct and indirect exposure to specific compounds compared to controls.
2. Experimental diets significantly increased survival of Atlantic salmon challenged by *P. perurans* compared to control diets.
3. Mucus samples collected from fish fed experimental diets had different characteristics compared to mucus collected from control fed fish.

Importance of nutrition on gill health and diseases

Rune Waagbø

NIFES, Bergen, Norway

There is a focus on gill health and diseases in farmed fish world-over, especially due to infectious diseases, like for example the amoebic gill disease (AGD) caused by the *Neoparamoeba perurans*, bacterial gill disease (BGD) caused by *Flavobacterium* sp. or fungi (*Saprolegnia*). However, other challenges may add to causes for poor gill health in farmed fish, like environmental pollutants and poor nutrition. The visual and physiological results are often similar, with increased mucus production, clubbed and fused gill lamellae, hyperventilation due to reduced respiration surface, as well as metabolic and osmoregulatory disturbances.

Recent changes in feed ingredients in aquafeed have called for more attention to individual nutrient supplies, both due to lower levels and bioavailability of nutrients in plant ingredients, and elevated nutrient requirements. Gill tissue is characterized by high metabolic activity (osmoregulation), oxygen exposure and delicate cell and blood vessel structures for gas exchange. Suboptimal nutrition of structural nutrients (lipids) and micronutrients (vitamins) can affect metabolism and cell integrity. Thus, suboptimal nutrition can affect both the gill tissue directly, as well as making the gills more prone to infectious agents.

The presentation will show results from previous micro nutrient studies and some initial results on the role of dietary lipids in gill health, as well as further plans to examine this relationship in an artificial challenge with AGD.

Snorkel cage barrier cage technology use and AGD infection

Lena Geitung, Daniel William Wright, Frode Oppedal, Lars Helge Stien, Egil Karlsbakk

Respiratory diseases are a huge cause of losses in farmed Atlantic salmon in Norway. *Paramoeba perurans*, which is responsible for amoebic gill disease (AGD), is one of the culprits of this rising concern. Recently developed lice barrier 'snorkel' cages, which consist of a standard cage fitted with a roof net to keep fish deeper and an enclosed tarpaulin tube (a snorkel) where salmon have access to the surface air used for filling their swim bladder while still avoiding surface waters where lice larvae are most abundant, have been reported to decrease salmon lice infestations without major impacts on salmon welfare. It has however, been reported that they increase the risk of AGD outbreaks. In three commercial scale studies, two with replicate snorkel compared to control cages and one including only snorkel cages, it has been demonstrated that snorkel cages increase the risk of AGD outbreaks. AGD outbreaks have been confirmed from qPCR and AGD-related gill scores. In all three studies the salmon in snorkel cages has experience an AGD outbreak in Autumn several weeks before the control cages. Snorkel cages have usually experienced a worse outbreak than control cages with higher AGD-related gill scores. Snorkel cages may increase the risk of AGD by excluding stock from intermittent brackish surface water linked to AGD reductions and intensifying crowding of stock. However, commercial-scale testing has also indicated that by establishing a limited volume of freshwater inside the snorkel, AGD related gill scores stall and eventually decrease. During one of the commercial scale studies (only with snorkel cages), high AGD-related gill scores were observed to decline when a freshwater surface layer was created inside the snorkel, for salmon to enter for self-treatment while jumping to re-fill their open swim bladder. Overall, the results suggest that snorkel technology increase the risk of AGD outbreaks, which may be circumvented by creating a freshwater layer inside the snorkel. Future investigations are on-going into freshwater snorkel and snorkel depth effect on infection dynamics of microparasites associated with gill disease focusing on *Paramoeba perurans* and AGD.

Key points of relevance/impact:
-Description of AGD infections in commercial 'snorkel' cages, one of the newly-developed preventive lice barrier cage technologies.
-Report of using a freshwater layer inside snorkels to control AGD, rather than relying on traditional bath treatments.
-Our research highlights the need to consider multiple parasite types when implementing a parasite control measure.

Scottish research priorities for gill health management

Robin Shields

Scottish Aquaculture Innovation Centre

Key findings are reported from a December 2016 industry-academia workshop that characterised recent trends in salmon gill health in Scotland and prioritised topics for research funding, as follows:

- Impacts of husbandry operations (marine and freshwater stages)
- Pathogen attributes, host-pathogen interactions and refinement of treatments
- Environmental impacts, monitoring and mitigation

The Scottish Aquaculture Innovation Centre (SAIC) has since issued a funding call and a series of multidisciplinary projects are currently being considered, spanning longitudinal epidemiological studies, development of gill health biomarkers, model-based decision support tools, and rapid detection of planktonic hydrozoans.

Opportunities for international engagement will be discussed.

POSTERS

Gill health in wild and farmed salmonids in British Columbia

Simon Jones

Department of Fisheries and Oceans Canada, Nanaimo BC Canada

Salmon aquaculture in British Columbia (BC) Canada is widely distributed on the Pacific coast and farmed salmon are exposed to a range of oceanographic and biological conditions. In addition, the industry co-exists with relatively large populations of anadromous Pacific salmon. The purpose of a new gill health research project is to document the distribution and severity of gill lesions in marine salmonids in British Columbia and to explore possible interactions of associated infectious agents between wild and captive salmon. This presentation will review factors known to be associated with gill disease in BC, review the recent outbreak of AGD and summarize available preliminary data.

AGD treatment strategies - Dose-response-studies with hydrogen peroxide and fresh water

Sigurd Hytterød[1], Linda Andersen[2], Haakon Hansen[1], Steffen Hageselle Blindheim[2], Jannicke Wiik-Nielsen[1] Trygve Thomas Poppe[4], Anja Bråthen Kristoffersen[1], Tor Atle Mo[4]

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Amoebic gill disease (AGD), caused by the amoeba *Paramoeba perurans* is considered a serious disease in the Norwegian salmon farming industry and there is a great need for the development of treatment strategies. Treatment with freshwater or hydrogen peroxide (H2O2) are the methods applied against AGD and both methods have proven to be effective against the amoeba.
Here we present the results from experimental studies where Atlantic salmon infected with *P. perurans* was treated with H2O2, fresh water and brackish water under environmental conditions relevant to Norwegian aquaculture. The effects of different H2O2 concentrations and exposure times were studied at different water temperatures to determine the H2O2 dose with the best reducing effect against AGD, while also being lenient to the fish. A further objective was to evaluate the effect of freshwater treatment against AGD at different water temperatures and exposure times.
The effects of the treatments were evaluated by following the development of AGD as macroscopic gill score and amoeba prevalence (real time PCR) during a period of 21 days post-exposure.
The bath treatments with fresh water had a better reducing effect against AGD compared to H2O2 treatments, and the fresh water treatments were significantly gentler to the fish. There was considerable variation in treatment efficacy when brackish water was applied. Treatments at low water temperatures and at low macroscopic gill score (early AGD stage) significantly enhanced a prolonged reducing affect against AGD.

There were no significant dose response correlations in the H2O2-treatments, neither from different concentrations nor from variation in exposure time. Treatment with H2O2 for 30 minutes or longer, however, led to gill bleeding, and in some cases significant fish mortalities, especially at water temperatures higher than 12 ^{0}C. Thus, when H2O2 treatments are applied, a short term exposure is imperative to safeguard fish welfare.

Histochemical characterization of AGD lesions in Ballan Wrasse (*Labrus bergylta*)

Herman Høgenes Kvinnsland[1], Henrietta Glosvik[2], Gordon Ritchie[2], Mark Powell[1, 3]
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Juvenile Ballan wrasse, *Labrus bergylta* were exposed to a polyculture of *Neoparamoeba perurans* trophozoites (1 hour at 1000 cells/L) in duplicate tanks maintained at 13oC in 34 ppt salinity. Control fish consisted of two tanks with un-exposed fish. Over the subsequent 6 weeks of maintenance, amoebic gill disease (AGD) only developed in the amoebae challenged group with a gross gill score peaking at 3 with 100% prevalence. Similarly, only the amoebae group showed characteristic AGD pathology and was the only group in which *Neoparamoeba peruans* were detected by PCR. Histology samples from the AGD exposed fish and the un-exposed fish were used for immunohistochemistry and other histological staining to examine and describe an AGD infection in Ballan wrasse. Staining methods used were AB/PAS, Giemsa and H&E, TUNEL was used to examine apoptotic cells. Using AB/PAS the mucous cells within defined inter-lamellar units were differentiated and counted. Mucous cells appeared to be more numerous in AGD live proliferative lesions and AGD affected fish than in healthy fish. Using Giemsa and H&E, granular cells (Eosinophilic Granular Cells, EGCs) were differentiated and appeared to have infiltrated the proliferative lesions of AGD affected fish but were more rare and sparse in the gill filaments of healthy fish.