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.

- Swabs - 4 weeks at 4°C
  - -20 for 6 month

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]

[1]Dept of Biology, University of Bergen, Thormøhlensgate 53B, 5006 Bergen Norway; [2] ILAB Industrilaboratoriet, Thormøhlensgate 55, 5006 Bergen Norway; 3 Norwegian Institute for Water Research, Thormøhlensgate53D, 5006 Bergen Norway.

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.

PATHOPHYSIOLOGICAL RESPONSES TO GILL DISEASE

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.

→ Environmental hypoxia occurs in sea cages
Johansson et al. 2006

→ - experiências em comparar resultado/capacidade de respiração de peixes doente

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.

Effects of AGD and Hypoxia on Survival and Metabolic Rate (paper)

Physiological pathogenesis of AGD – AGi

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

Cyanea capillata — jellyfish
- increase in cardiac output
- vascular changes
- homorrhages
- change in breathing pattern

Amoeba

delays AGD development → kind of protection

→ cardiac toxine
→ neurotoxine
→ hemolitoxine

Ectopleura phaye and amoeba
→ exposure to hydroides caused associated

AGD and hypoxia tolerance
PRV - infection seems reduced tolerance to hypoxia

PRV - infection is highly prevalent in Norway

- Development of lesions on the gills limit CO_2 excretion
- High DAP and systemic vascular resistance (Atlantic Salmon)
- Cardiac remodelling as consequence of vascular resistance
- Blood pH in AGD is consistently reduce (Acidosis)
- AGD fish antes de verem sinais clínicos eles são alcalóticos (mm antes de doenças tornarem a doença) - hyperventilation reaction and acid-base metabolic reaction

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.

Bacterial Analysis — Snap
16SRNA - Test pour conséquences ? vacuoles

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.

CRESCIMY VS AGD SCORING - CORRELATES NEGATIVELY

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]

[1]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA, United Kingdom.; [2]Ridgeway Biologicals Ltd., Old Station Business Park, Compton, RG20 6NE, United Kingdom;[3]Marine Harvest Scotland, Blar Mhor Industrial Estate, Fort William, PH33 7PT, United Kingdom.

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

Free in the sediment under net pens

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]

[1]Norwegian Veterinary Institute; [2]Industrial and Aquatic Laboratory; [3]Pharmaq Analytiq; [4]Norwegian Institute for Nature Research.

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 °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]
[1]*University of Bergen, Bergen, Norway;* [2]*Marine Harvest AS, Bergen, Norway;* [3]*Norwegian Institute for Water Research, Oslo, Norway*

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.

1°. Not great numbers to put as not notifiable
- prod. of Norwegian
- fish health report - gives some data/private laboratories
  - Loquest Back-survey - FH services (37 participi)
    NFSS Authority
    - Survey in will be always to map the prevelance of Paxvirus
- AGD - Detected qPCR - all over
  - Southwest coast most affected/farmers control the disease quite well and the gill scoring and then treat.

Multifactorial disease causing problems (west coast)
Not then less affected

Salmonid Pox virus - 11 smolt production
  - farms (causes ↑ mortality in same sites)
  - not so problem in seawater but ↑ in freshwater

Prevalence
- Wild salmonid → Detected in 25 of 26 watercourses (4 species)
  - Anadromic Atlantic salmon
  - ↑ prevalent
  - seatrout also - Broodfish kept together / The kept together were more prevalent than the seatrout kept alone*
  - laudlock trout
- * so seatrout not natural host

Braunchiomonas cysticola - FW and SW usually in mixed infections / Problem in some FW farms with RAS / Not always produce a response to it

Desmozoon - Both gills and other organs / intranuclear
mixed gill-infections / FW farms in 2015 and 2016/
emerging threat (smolt production threat)

Algal blooms and jellyfish - not assessed to be a problem of concern 2016.

2° talk - Monitoring and diagnosis (Included fishvetgroup)
- cost of treatments (Δ 3 treatment/per cycle (90% FW and 10% H_2O_2)
- AGD more under control but ? incidence of other situations
- Δ wellboats/FW ⇒ late treatments
reduced doses

Wrasse/lumpfish - looks ≠ AGD and it's easy to miss / Recommend swabs. / fresh water treat as soon as possible

Biofouling damage to fish

3ª talk - Ester abstract

4ª talk - Australia perspective

5ª talk - Chile update (Fishvetgroup)

6ª talk - MH Health update

Parvives - in FW sites

Brachiomonas

Behaviour/PCR/Feeding

cleaner fish positive ⇐ salmo negative

cohabiting fish is not always treated for AGD

7º talk - Abstract POMAs

8º talk - Gill disease PHARMAQ Analytiq AS (tauho & serz & data (hadas uce decmicag)

9º talk - Barbara Nowak

Discussion

Comment: anorexia observed in MH (D. Cockrill)

" " Fishvetgroup Scotland seen too but no explanation.

- subclinical stuff IMP (Hal comment) - Biofilm can have effect on some days/weeks after.
- netcleaning Role on gill (not known) - comment gap.
- netchanging - can be time cost / Link between FW/SW

Afternoon talk [illegible]

1° talk Are Nylund

- what are the most imp. Risk factors for CGD?
- How can we control the risk factors
- Do we have basic knowledge to control the risk factors
- if not enough knowledge what we would sugest to fill this gaps?

salmon biology - como se faz nos outros animais/indicadores de saúde/patogenes são end points.

Swabs - sensitivit to AGD/Bcys/Pox/Pthe (less sensitive bc deep inside)

Advantages

Sensitive: AGD and Pox - more sensitive than gill tissue sample

non-lethal
- fish

Easy - can be done when counting lice/gills coring
- less equip.

Swabs transported in media

Karin Pittman

live cells at the surface of fish skin - mucous is important as a barrier
mammals is queratinized
- anti-fungus
- anti-bact

H_2O_2 - causing irritation oesophago - ↑ mucous cells → lost of apetito.

Wild had shorter lamellae
No mucous cells in lamellae | Tret et al. 2002.
- lifting [illegible] of gill epithelio

$-H_2O_2$ = oedemas
lamellar fusion
epithelial hyperplasia and swelling

Healthy gill lamellae have few mucous cells - objective and quantifiable

MARK Powell - 2nd day

Fw bathed - gives a physiological benefits/ removes AGD and improves swimming.

Gill score 2 threshold point to start seeing physiological problems.

Mitchell paper 12% - scoring histology for AGD

Amoeba and bacteria - Siliquasuo