WORKSHOP REPORT

Marine Gill Histopathology Workshop

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Introduction
A histopathology workshop took place as part of the Gill Health Initiative (GHI) at the University of Stirling, Scotland on January 30th 2013. The workshop was aimed at those who use histopathology as a diagnostic tool or in research applied to gill health. There were 38 attendees with clinicians or pathologists from Australia, Faroe Islands, France, Ireland, Norway, Scotland and Spain all represented. The majority of cases presented were of gill disease in marine Atlantic salmon (*Salmo salar*), but cases from species such as sea bream (*Sparus aurata*), seabass (*Dicentrarchus labrax*) and turbot (*Psetta maxima*) were also examined.

Aims
The aims of the workshop were primarily to clarify the criteria being used in the diagnosis of *Amoebic Gill Disease* (AGD), microsporideal gill disease as associated with *Desmozoön lepeophtheri* (syn. *Paranucleospora theridion*) and any other interesting gill conditions that had been encountered by the clinicians and researchers present at the workshop. It was hoped that one of the outcomes of the workshop would be agreement on the descriptions of the diagnostic criteria for each of these diseases between (and within) countries. We felt that it was important to clarify these diagnostic criteria for a number of reasons:

a. Diagnosis: correct aetiologies, prognosis, gill disease treatments, decisions on sea lice treatments, etc.
b. Epidemiology & research
c. International communication & research
d. To identify gaps & work required

Hamish Rodger of Vet Aqua International opened the workshop with an introduction of the aims and current criteria (or lack of) for gill conditions in diagnostic histopathology. The main points were:

e. Histopathology remains one of the most important diagnostic tools in aquaculture and is the gold standard for many disease conditions.
f. There is a need to clarify the diagnostic criteria in relation to proliferative gill diseases.
g. Histopathology associated with harmful algal blooms or zooplankton swarms requires better

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Amoebic Gill Disease (AGD)
Cases were presented from Scotland (Randolph Richards, Marianne Pearson, David Cox, Mar Marcos-Lopez), Australia and New Zealand (Mark Powell) in Atlantic and chinook salmon, and from the Mediterranean in sea bass, turbot and sea bream (Francesc Padros, Albert Girons). The diagnostic criteria were relatively clear for AGD and were agreed as follows:
- hyperplasia plus
- lamellar fusion plus
- presence of vesicles plus
- presence of amoebae (with the characteristic eosinophilic parasite on H & E) (some may be obvious in the vesicles)

These criteria are consistent with the case definitions previously given (Clark and Nowak, 1999; Munday et al. 2001) for AGD in Tasmania and additional observations for cases observed included oedema and pavementing of squamous epithelium and in some cases in Europe multifocal liver necrosis.

The following observations were made during the discussion:
- The importance of sampling strategy was noted, as AGD could be very focal and frequently normal gill tissue can be found adjacent to areas of severe pathology.
- Orientation of slides can be important to get an accurate idea of the hyperplasia present in the gills.
- Davidson’s fixative can be used as an alternative to formalin for fixation of gills with amoeba, however, for the routine processing of samples in diagnostic work it may not be a practical alternative due to the requirement of removing acetic acid before using samples in a closed-system tissue processor.
- Alcian blue is a good stain for amoebae but also stains the mucous cells in the gill tissue.
- Research has shown that previously described lacunae that contain the amoebae are enclosed vesicles (Adams and Nowak, 2001).
- The debate arose as to whether a case should still be classified as AGD post-treatment, when all the parasites were removed but the pathology remained. The general consensus was that as gills affected with AGD post treatment can look similar to other clinical gill diseases it is important to specify the previous clinical history when describing the histopathology.
- Gill cartilage pathology and other aberrations were occasionally observed and the cause remains to be determined.
- AGD in Mediterranean species is frequently complicated by *Tenacibaculum* spp. infection. AGD is not as clinically significant in these species in terms of mortality and loss of growth as it has been in Atlantic salmon.
Other hyperplastic or proliferative-type gill diseases
Cases were presented from Norway, Scotland and Ireland (Mona Gjessing, Kai-Inge Lie, David Cox, Sara Pflaum, Susie Mitchell) in Atlantic salmon. In Norway the diagnostic criteria for proliferative gill inflammation or PGI have been described (Kvellstad et al., 2005) as:
- Hyperplasia plus
- Cell death (necrosis) plus
- Circulatory disturbances (haemorrhages) plus
- Inflammation
- +/- presence of epitheliocystis, parasites and other infectious agents.

However, it was discussed whether these criteria were too vague to be useful and may actually be a description of end-stage pathology from a variety of challenges and/or pathogens. Very few cases of gill disease with the criteria of PGI were reported in Norway in 2012, however, other gill disease problems were observed. Mona Gjessing (MG) presented a typical case of PGI where the pathology included hyperplasia, inflammation, necrosis and haemorrhage (Case 11). Epitheliocystis, Ichthyobodo (costia) and Trichodina are also present in some fish. The second gill case MG presented had extensive proliferative pathology but didn’t meet all the criteria listed for PGI. Kai-Inge Lie presented three cases with proliferative-type gill pathologies. The main histology findings were hyperplasia, fusion, necrosis and various types of vascular pathology (thrombi, telangiectasis). The gill pathology was accompanied in one case by severe liver necrosis. High levels of epitheliocystis were observed in two of the cases (Case 12).

Cases of a proliferative-type gill disease were presented from Scotland in Atlantic salmon by David Cox and Sara Pflaum. Typical pathology included hyperplasia, fusion, inflammation and circulatory disturbances, the presence of ballooning necrosis in epithelial cells, +/- the presence of the microsporidian Desmozoon lepeophtherii (syn. Paranucleospora theridion) in the cells (which stain strongly Gram positive when retained spore material present or Gram negative if just the microsporidea shells remain), +/- amoebae and +/- epitheliocystis. This histopathology has been considered as associated with a distinct autumn proliferative gill disease in Scotland. The pathology described was similar to the criteria for the proliferative gill pathologies described from Norway. A new paper in press at time of writing describes these findings in Scotland (Matthews et al., 2013).

Summary of proliferative-type gill diseases
It became clear during the workshop that PGI in Norway, proliferative gill disease in Scotland and other presentations of proliferative-type gill pathologies shared histopathological similarities. There was considerable overlap in the histopathological changes across all the cases presented. As the gills have a limited range of responses to injury it can be challenging to make a clear diagnosis based on the pathology. The term proliferative gill disease (PGD) is in established use in the USA for a well described catfish disease associated with the myxozoan parasite, Henneguya sp. (Lovy et al., 2011; Noga, 2010) , so it is suggested that this term is avoided for salmon to prevent confusion.
Further work is required on the cause/s of these proliferative-type gill diseases and with various research projects underway it is proposed that a follow-up workshop be held (possibly to coincide with the next GHI meeting in 2014) to provide a forum for further discussion and clarification on these. Other areas that remain as gaps in our knowledge or that require to be addressed include:

a. Histopathology and pathogenesis of gills exposed to specific harmful algae species (with or without additional pathogen involvement) (there has been no published work on challenge experiments with harmful marine algae)

b. Histopathology of gills exposed to fouling organisms (hydroids, etc.) (there have only been limited studies undertaken on exposure of fish to hydroids (Baxter et al., 2012), and none with hydroids plus gill pathogens)

c. Histopathology of gills exposed to chemical or medical remedies

d. Experimental work is required on the pathogenesis of challenge with gill pathogens such as the microsporidian *D. lepeophtherii* and the bacteria *Candidatus* Branchiomonas cysticola.

e. Further, it was highlighted that the terminology used in gill histopathology requires some revision (and in some cases correction).

**Selection of cases presented at the gill disease workshop**

Case 1: Shetland Islands, Scotland, AGD in seawater Atlantic salmon. Large variation in gill pathology was observed from fish to fish, and from gill arch to gill arch. Severe hyperplasia and fusion with extensive vesicle formation and several amoebae within the vesicles were observed. Oedema and spongiosis was present throughout the epithelium. Pavementing of epithelium (a squamous layer of epithelium on the surface of the lamellae) was observed in mature lesions, frequently in association with high numbers of amoebae. This pathology was observed adjacent to areas of normal gill tissue (Randolph Richards).
Case 2: Scotland, seawater Atlantic salmon. These were 2012 S0s after three weeks at sea. Clinical AGD was present on 1/5 fish from routine sampling. The lesion was extremely localised and involved the base of two primary lamellae. Extensive ballooning spongiosis was observed with the surface of the affected lamellae covered with amoebae (Marianne Pearson).

Case 3: Scotland, seawater Atlantic salmon. This case was from 2011 S0s. The fish started with clinical AGD (August 2012) and were treated 3x with hydrogen peroxide (1400mg/l, 20 minutes). There was a subsequent progression in the pathology observed. The AGD lesions became chronic and the amoebae numbers declined. This was accompanied by an increase in inflammatory foci, haemorrhage, congestion and necrosis and appearance of the microsporidian (D. lepeophtherii) with ballooning necrosis of the epithelial cells (with microsporida present in these ballooning necrotic cells) and epitheliocystis, culminating in a diagnosis of proliferative-type gill disease in December (David Cox).

Case 4: Scotland, seawater Atlantic salmon. Severe case of AGD with very high numbers of amoebae observed with extensive fusion of the gills (Mar Marcos-Lopez).
Case 5: Scotland, seawater Atlantic salmon. Treatment damage post hydrogen peroxide was observed in these fish with AGD characterised by karyorrhexis, oedema, epithelial separation, necrosis, vascular damage and shrinking of surface epithelial cells (Mar Marcos-Lopez).

![Image of histological sections showing vascular damage and shrinking of surface epithelial cells](image1.png)

Case 6: Scotland, seawater Atlantic salmon. Severe AGD with concurrent *Ichthyobodo (costia)* infestation (Mar Marcos-Lopez).

![Image of histological sections showing AGD with *Ichthyobodo (costia)* infestation](image2.png)

Case 7: Spain, AGD in turbot. This is an early lesion from a typical case of AGD in turbot. Fusion of the lamellae causing vesicle formation. Amoebae can be observed within these vesicles alongside mats of *Tenacibaculum* sp. (Francesc Padros)

![Image of histological sections showing AGD in turbot](image3.png)
Case 8: Scotland, seawater Atlantic salmon. 2012 SI’s and proliferative-type gill disease was observed in these fish in their first autumn at sea. Left: Typical ballooning necrosis that is frequently observed with microsporideal infection can be observed in this section. Epitheliocystis can also be observed in the distal lamellae. Right: Gram-Twort stain to illustrate the microsporidea (D. lepeophtherii) spores in an area of ballooning necrosis (Sara Pflaum).

Case 9: Ireland, freshwater Atlantic salmon. Typical presentation of epitheliocystis in freshwater Atlantic salmon (Mitchell et al., 2010). Colonies of ‘Candidatus Clavochlamydia salmonicola’ can be observed between the secondary lamellae without any associated pathology. These freshwater epitheliocystis infections can persist in seawater for a few weeks post transfer so can be seen occasionally in seawater Atlantic salmon (Susie Mitchell).

Case 10: Ireland, seawater Atlantic salmon. Two pictures with different presentations of epitheliocystis. Left: Typical cysts seen in Atlantic salmon in the lamellar tips with very little pathology surrounding the bacterial colonies. Right: Multiple bacterial colonies in highly proliferative gill tissue. These fish had severe AGD a few months prior to sampling. At the time of sampling there were no amoebae remaining. Extensive proliferative pathology was present, with high numbers of cysts characteristic of ‘Candidatus Branchiomonas cysticola’ (Toenshoff et al., 2012) (Susie Mitchell and Hamish Rodger).
Case 11. Norway, seawater Atlantic salmon. Histopathology of gills presenting with hyperplasia, necrosis, focal haemorrhages and some inflammatory cells, as previously described in proliferative gill inflammation (PGI) (Mona Gjessing)

Case 12. Norway, seawater Atlantic salmon. Gills from salmon which were swimming with gaping mouths and fish farm had increasing mortality. Grossly gills appeared swollen and pale and livers were mottled. Histologically there was hyperplasia, fusion, necrosis and various types of vascular pathology (thrombi, telangiectasis). High levels of epitheliocystis were observed in one fish (right image). One fish also had severe liver necrosis (Kai-Inge Lie).
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References


