Final Report

Atlantic Salmon Aquaculture Subprogram: The effects of AGD on gill function - use of a perfused gill model

Melanie J. Leef, Barbara F. Nowak

FRDC PROJECT NUMBER: 2011/069

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Atlantic Salmon Aquaculture Subprogram: The effects of AGD on gill function - use of a perfused gill model

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2011/069 Atlantic Salmon Aquaculture Subprogram: The effects of AGD on gill function - use of a perfused gill model

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OBJECTIVES:
1. Development of an in vitro perfused Atlantic salmon gill model for use in both AGD and non-health based physiological studies
2. In vitro assessment of gill function in AGD-affected fish
3. Investigation of altered gill blood flow patterns and branchial (gill) vascular resistance in AGD-affected fish

Non-Technical Summary

OUTCOMES ACHIEVED

This project has successfully adapted an isolated perfused gill model specifically to Atlantic salmon and has provided in vitro results of gill function and branchial vascular resistance in AGD-affected animals. This model represents a novel in vitro method for AGD research

A protocol for preparing the gill arch and subsequent measurement of oxygen consumption (MO_2) was developed and the optimum time for experimentation was determined to be within 3 hours of gill placement within the respirometry chamber. Gill arches from fish less than 700g were not suitable for use due to small vessel size which led to cannulation difficulties. Results for the examination of gill function in experimental fish were similar to those reported in the literature. Investigation of gill function in AGD-affected fish found there was a negative relationship between percentage of affected filaments and MO_2. Results following the addition of the sodium-potassium adenosine triphosphatase (Na^+K^+ATPase) blocker to the gill perfusate suggested oxygen costs associated with osmoregulation are lower in AGD-affected gills. This result is most likely a consequence of the hyperplastic AGD lesions which effectively fill the non-respiratory areas of the gills where the majority of chloride cells reside. Elevated branchial resistance was also noted in AGD-affected animals.

Respirometry: Technique used to estimate metabolism
Cannulation: The insertion of a cannula into a vessel
Hyperplastic: An abnormal increase in the number of cells in an organ
Branchial: Of or relating to the gills
Chloride cells: Ion transporting cells in the gills of fish

The primary end users and beneficiaries of this project are the TSGA, other researchers and the project investigators. While the benefits of this project are primarily non-pecuniary, the knowledge generated using this model could contribute to the elimination of AGD costs (approximately $15M). Successful mitigation of AGD will result in substantial reduction of health related costs both direct (labour, infrastructure, freshwater) and indirect (lost growth, mortality). Lower production costs will lead to more cost effective production and a more profitable and sustainable industry.

The results within this report are currently in preparation for publication in peer reviewed scientific journals. These publications will provide further dissemination of this research and will directly benefit the PI and CI. Additionally publication of the research methods and the subsequent application of the in vitro perfused gill model to other health and non-health physiological studies will benefit other researchers. As well as publication results of this project have been regularly communicated to the TSGA chief executive officer and the FRDC over the duration of the project in the form of milestone reports and informal discussions. Results have also been presented by the project PI at a workshop on fish parasites presented at the Australian Society of Parasitology: Parasitic Diseases in Fish Mariculture Workshop (Feb 2013) and the FRDC Australasian Aquatic Animal Health Conference (July 2013).

KEYWORDS:
Atlantic salmon, AGD, in vitro gill model, gill function, branchial resistance

Acknowledgments

This study formed part of salmon industry research, and was supported by the Fisheries R&D Corporation. We would like to thank the salmon industry for their collaboration and support particularly Van Diemen Aquaculture.

Background

Amoebic gill disease (AGD), caused by the pathogenic free living protozoan *Neoparamoeba perurans* is the most significant health issue currently affecting the production of Tasmanian Atlantic salmon *Salmo salar*. In general the most severe AGD outbreaks occur during the summer and epidemiological investigations have positively correlated outbreaks with both increasing temperatures and salinity (Munday et al., 1993; Clark and Nowak, 1999; Nowak, 2001; Douglas-Helders et al., 2001; Adams and Nowak, 2003). The current industry practice for treatment of affected fish is bathing in freshwater. Due to the high economic costs associated with AGD management, mitigating the effects of this disease is a primary concern for industry.

Although significant research outcomes have been achieved in understanding the pathophysiological effects of this disease, identification of the actual physiological mechanism/s for AGD related mortality have remained elusive (Powell et al., 2002; Leef et al., 2005ab; 2007abc). Both respiratory distress and lethargy have been documented in farmed AGD-affected salmonids (Kent et al., 1988; Munday et al., 1990; Rodger and McArdle, 1996). Additionally with gross signs of infection limited to the gill, it was presumed that mortality was associated with respiratory failure. Subsequent in vivo physiology based studies however, including hypoxic challenge experiments, failed to show hypoxemia (low blood oxygen) as the primary cause of mortality. Acid-base disturbance and cardiovascular dysfunction have since been suggested as more likely candidates (see Powell et al., 2008). Most of the previous physiological studies were highly reliant on the use of lightly affected animals as heavily affected fish were unable to survive the necessary surgical and/or experimental procedures. The difference between heavily affected (and moribund) animals and those with ‘light’ AGD are not known, therefore the issue of respiratory failure as the mechanism for AGD-related mortality currently remains unresolved.

Application of an in vitro gill model specifically for Atlantic salmon would negate the need for lightly affected fish. Isolated perfused gill models have been widely used by researcher for more than 80 years to investigate a myriad of physiological mechanisms and processes including gas transfer, ion exchange and haemodynamic patterns (Lyndon, 1994; Morgan and Iwama, 1999; Deigweilher et al., 2010). Additionally these models have successfully been used in other disease related pathophysiological studies and also to examine host pathogen interactions (Decostere et al., 1999ab; Tobback et al., 2010; Nematollahi et al., 2003). An in vitro model for AGD research such as a

Pathogenic: Infective, able to cause disease
Pathophysiology: Study of the functional changes associated with or resulting from disease or injury
In vivo: Taking place within a living organism
In vitro: Taking place outside of a living organism
Moribund: Dying,

perfused salmon gill model would allow the physiological mechanisms relating specifically to gill function to be quantified for heavily affected and moribund animals. Understanding the physiological effects of AGD in heavily affected animals could provide the key to the actual mechanisms by which this disease causes mortality. This in turn would provide alternative and novel avenues for research into AGD treatments that are directed towards host that could help to mitigate the effects of disease.

Need

With a production value of over $362 million (2009-2010 ABARE), the Tasmanian Atlantic salmon industry is now the most economically valuable finfish industry in Australia. This industry employs over 2000 people therefore the social, economic and arguably cultural values to the Tasmanian community are considerable. AGD is the most significant health issue affecting the production Atlantic salmon in Tasmania with estimated costs to the industry of up to 20% of total production. These costs relate to freshwater bath treatments, which require specialised infrastructure and are labour intensive, as well as lost production through both mortality and decreased growth. AGD outbreaks are more frequent in the summer months however with increasing temperatures bathing frequency and associated costs appear to be extending into *t*raditionally AGD-free periods such as spring and autumn. Mitigation of these costs is therefore essential to maintain the profitability of the industry.

The in vitro perfused gill preparation offers a novel approach to AGD research. Manipulation of the model to varying external (surrounding medium) and internal (perfusate) conditions will allow direct examination of gill function in not only AGD-affected fish but also has direct application for other health and non-health based physiological studies.

Objectives

1. Development of an in vitro perfused Atlantic salmon gill model for use in both AGD and non-health based physiological studies

2. In vitro assessment of gill function in AGD-affected fish

3. Investigation of altered gill blood flow patterns and branchial (gill) vascular resistance in AGD-affected fish

Methods

Development of an in vitro perfused Atlantic salmon gill model for use in both AGD and non-health based physiological studies

Fish

Seawater Atlantic salmon *Salmo salar*, of approximately 400-1000g, were transported from Van Diemen Aquaculture to the University of Tasmania on the 5th May 2012. On arrival fish were transferred to a 4000l recirculating system and held at 20ppt and 13°C. Following an extended period of recovery fish were acclimated to 35ppt and 15°C for experimental purposes. Fish were offered feed daily and water changes were performed when necessary. Water quality parameters including temperature, salinity, pH, ammonia (NH_4), nitrite (NO_2) and nitrate (NO_3) were measured at least weekly.

Experimental design

Individual arches collected from individual fish were perfused over the course of 60-180 mins. Viability of the preparation was assessed histologically from arches fixed at 60, 120 and 180 min post perfusion. For comparison, non-perfused arches held in saline were similarly fixed at 0 (control), 60, 120 and 180 min. In addition to histology, oxygen consumption of the preparation within the chamber was used to assess viability.

To assess long term viability individual preparations were perfused over a 20+ h period according to the method of Deigweilher et al., (2010).

Perfused/isolated gill preparation

Preparation of gill arches was performed according to Morgan and Iwama (1999). Individual fish were anesthetised with 20mg/l Aqui-S, weighed and then injected via the caudal vessels with heparinised (5000 U/Kg fish) Cortland's marine saline (see Table 1 for saline composition). The fish was then returned to anaesthetic bath for at least 5 min before the anaesthetic solution was increased to a lethal dose of 40mg/l. Following euthanasia a midline ventral incision was made to expose the heart and the tail was removed. The ventricle was pierced with a blunt 23G needle connected to a 30cm length of PE50 tubing and perfused with heparinised (10U/ml) saline until the gills were cleared of blood (Fig. 1). The entire gill basket was then removed and the first left gill arch was excised and placed into ice cold saline for cannulation. The second left gill arch was excised and immediately placed into seawater Davidson's fixative solution for histology. The remaining gill arches were separated and similarly placed into saline for fixation at 60, 120 and 180 min (non-perfused histological controls).

Table 1. The chemical composition of Cortland's marine saline. Prior to use the saline was 0.2µm filtered.

Chemical	mM
Sodium chloride (NaCl)	139
Potassium chloride (KCl)	5.1
Calcium chloride ($CaCl_2$)	1.1
Magnesium sulphate ($MgSO_4$)	0.9
Sodium bicarbonate ($NaHCO_3$)	11.9
Monosodium phosphate (NaH_2PO_4)	3.0
Glucose	5.6
pH 7.5	

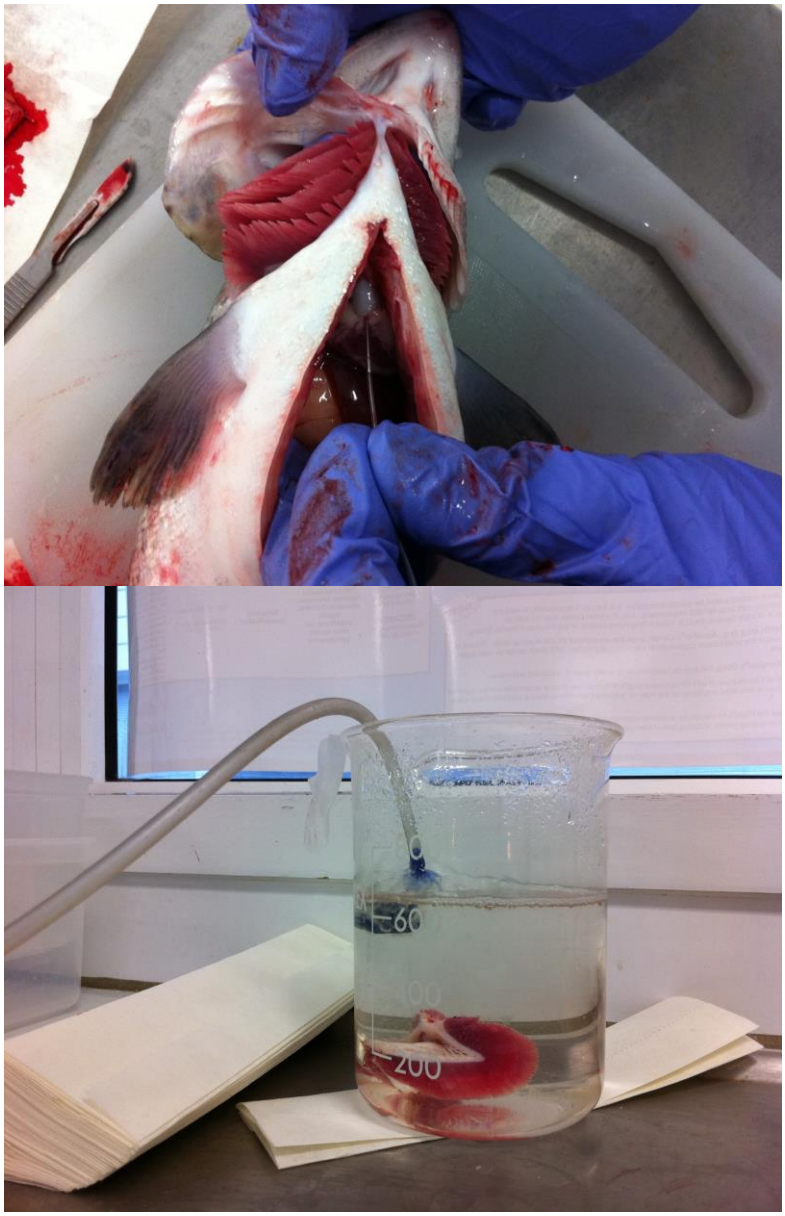

Figure 1. Perfusion via the ventricle to clear the gills of blood prior to removal of the branchial basket (shown below in ice cold saline) and subsequent cannulation of an individual arch.

Cannulation of the isolated gill arch was performed according to Morgan and Iwama (1999) with minor changes. In brief the afferent (ventral) and efferent (dorsal) arteries were cannulated with saline filled PE50 tubing that was slightly tapered at the cannulation end. Each cannula was secured in place using a cyanoacrylate tissue adhesive (Vetbond 3M). Silk suture was then used to occlude the remaining vessels at each end of the arch. Vetbond was also used to seal the ends of the arch (Fig. 2).

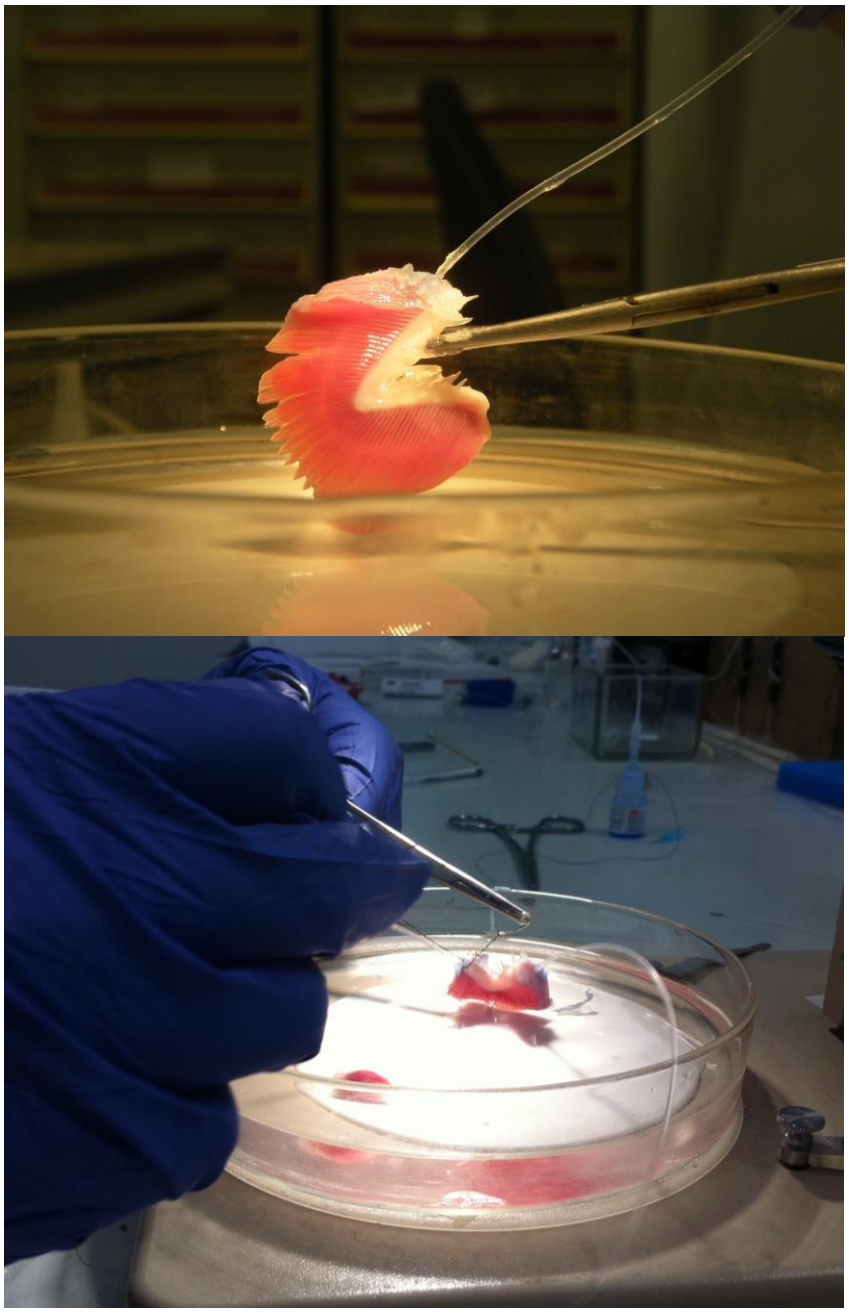

Figure 2. Cannulation of the afferent branchial artery. The tapered end cannula was held in place using a cyanoacrylate tissue adhesive (Vetbond). Each cannula was additionally secured with silk sutures.

Experimental setup

Components of the experimental setup are shown in Fig. 3. Individual preparations were suspended in a glass chamber containing pre aerated 0.2µm filtered seawater 35ppt. The glass chamber was sealed and each cannula was attached, via 23G tubing connectors, to flow through afferent and efferent O_2 electrodes that were used to monitor oxygen content of the perfusate. A magnetic stirrer was used to facilitate gas diffusion and to maintain consistent gas mixture within the water bath. The preparation was perfused with a pulsatile flow of saline using a peristaltic pump. Oxygen electrodes (2 x flow through and 1 x needle monitoring O_2 content of the external medium within the glass chamber) were calibrated with air saturated saline (flow through) or seawater (needle) and a 2% sodium sulphite ($NaSO_3$) zero solution. Monitoring of O_2 was continuous over the perfusion period using a PowerLab system and Chart 4.0 software.

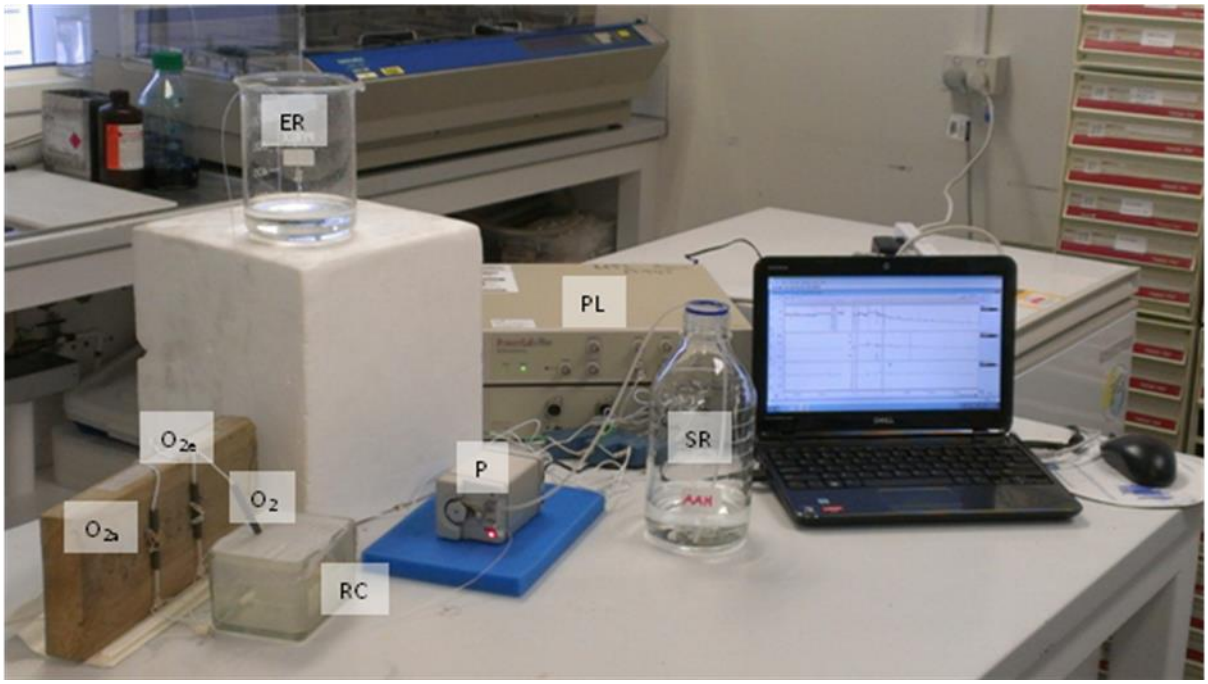

Figure 3. Experimental setup showing saline reservoir (SR), peristaltic pump (P), afferent flow through O_2 electrode (O_{2a}), glass respiration chamber (RC), needle O_2 electrode (O_2), efferent flow through O_2 electrode (O_{2e}), effluent reservoir (ER). A PowerLab data acquisition system (PL) and laptop computer was used to continuously record O_2 measurements using Chart 4.0 software. Note that the peristaltic pump was placed on a foam pad in order to reduce vibrations that were found to interfere with steady electrode measurements.

In vitro assessment of gill function in AGD-affected fish

Fish

Seawater Atlantic salmon *Salmo salar*, of approximately 1000g, were transported from Van Diemen Aquaculture to the University of Tasmania and

transferred to a 4000l recirculating system and held at 20ppt and 13°C. Following an extended period of recovery fish were acclimated to 35ppt and 16°C for experimental purposes. Fish were offered feed daily and water changes were performed when necessary. Water quality parameters including temperature, salinity, pH, NH_4, NO_2 and NO_3 were measured at least weekly.

AGD infection
AGD-affected fish (n=10) in this study were infected via cohabitation for at least 2 weeks prior to gill preparation. Disease status was confirmed histologically.

Perfused/isolated gill preparation
All gills were prepared according to the final protocol outlined above.

Experimental design
Individual arches n=2-3 collected from individual fish (AGD-affected and non AGD-affected) were perfused over the course of 180 mins according to the above protocol. Leaking preparations were discarded. Viability of the preparation was assessed histologically from arches fixed 180 min post perfusion. In addition to histology, oxygen consumption of the preparation within the chamber was used to assess viability.

Statistical analysis
Comparison of mean *MO*2 in AGD-affected and non AGD-affected gill preparations was assessed using an independent t-test.

Investigation of altered gill blood flow patterns and branchial (gill) vascular resistance in AGD-affected fish

For investigation of branchial vascular resistance pressure transducers were placed on both the afferent and efferent side of the gill preparation. Pressure recordings were made for the period of experimentation for both AGD-affected and non-affected control gills.

Results/Discussion

Development of an in vitro perfused Atlantic salmon gill model for use in both AGD and non-health based physiological studies

Histology

Histological examination of perfused and non-perfused gills showed that gill structure and morphology was relatively well maintained in both perfused and non-perfused gills over 180 min (Fig. 4). Individual lamellae were clearly defined even up to 180 min post perfusion/non perfusion. Filaments and lamellae were still identifiable in the arches left overnight however degeneration of gill tissue was evident.

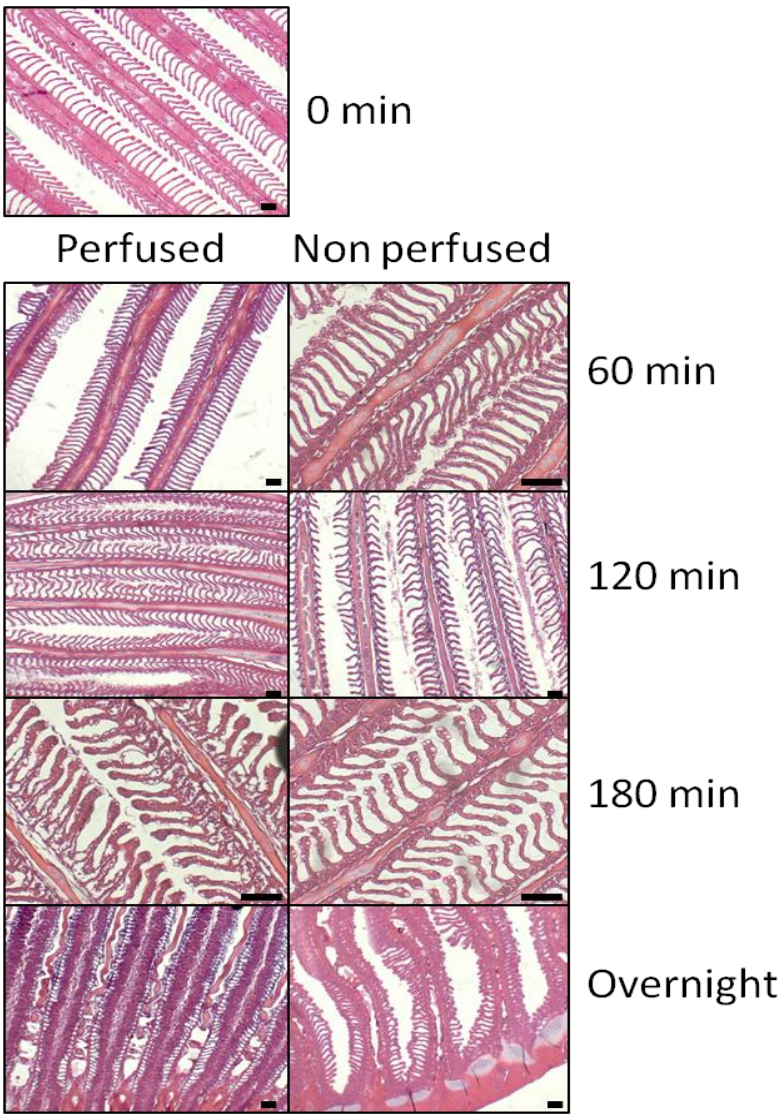

Figure 4. Histological sections from perfused gill arches that were fixed in seawater Davidson's at 60, 120, 180 min post perfusion. Non-perfused arches were maintained in saline until fixation at each time point. The 0 min control was fixed immediately following the removal of the branchial basket. Scale bars = 100µm.

O_2 consumption and assessment of viability

Results from the continuous recording of O_2 over a period of 20+ h showed that all of the O_2 within the respiration chamber was consumed within 13 h (Fig. 5). This suggests that the current model is not suitable for long term experimentation however larger chamber/respirometer volumes or supplementation of additional O_2 within the chamber could potentially extend viability. Comparison of data recorded in the blank chamber not containing a gill preparation however clearly demonstrates the viability of the isolated gill to readily consume O_2 (Fig. 6).

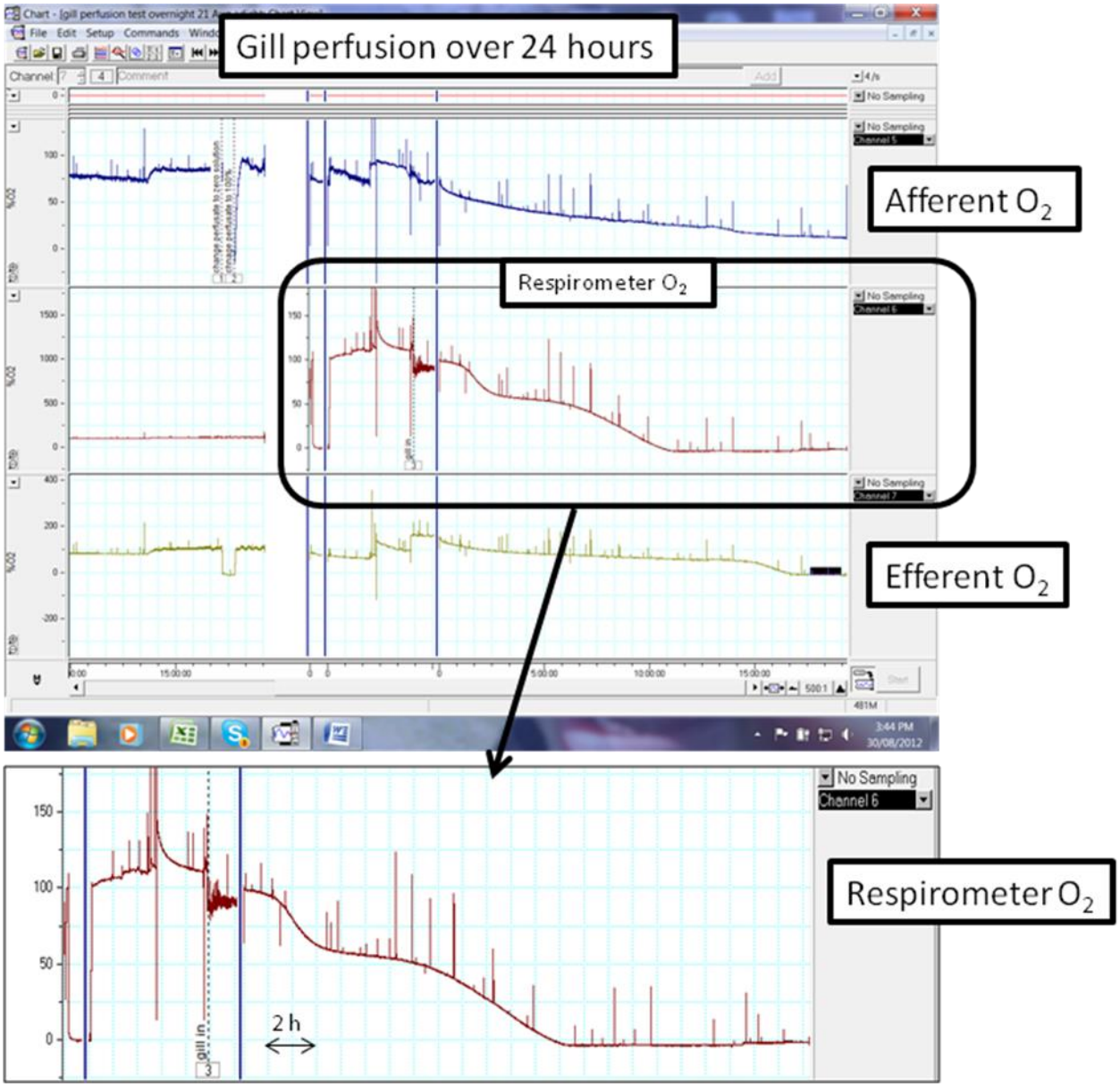

Figure 5. O_2 (%) recorded continuously over 20+ hours in afferent saline, seawater (gill chamber/respirometer) and efferent saline following the addition of a cannulated gill preparation. Time scale in hours (h)

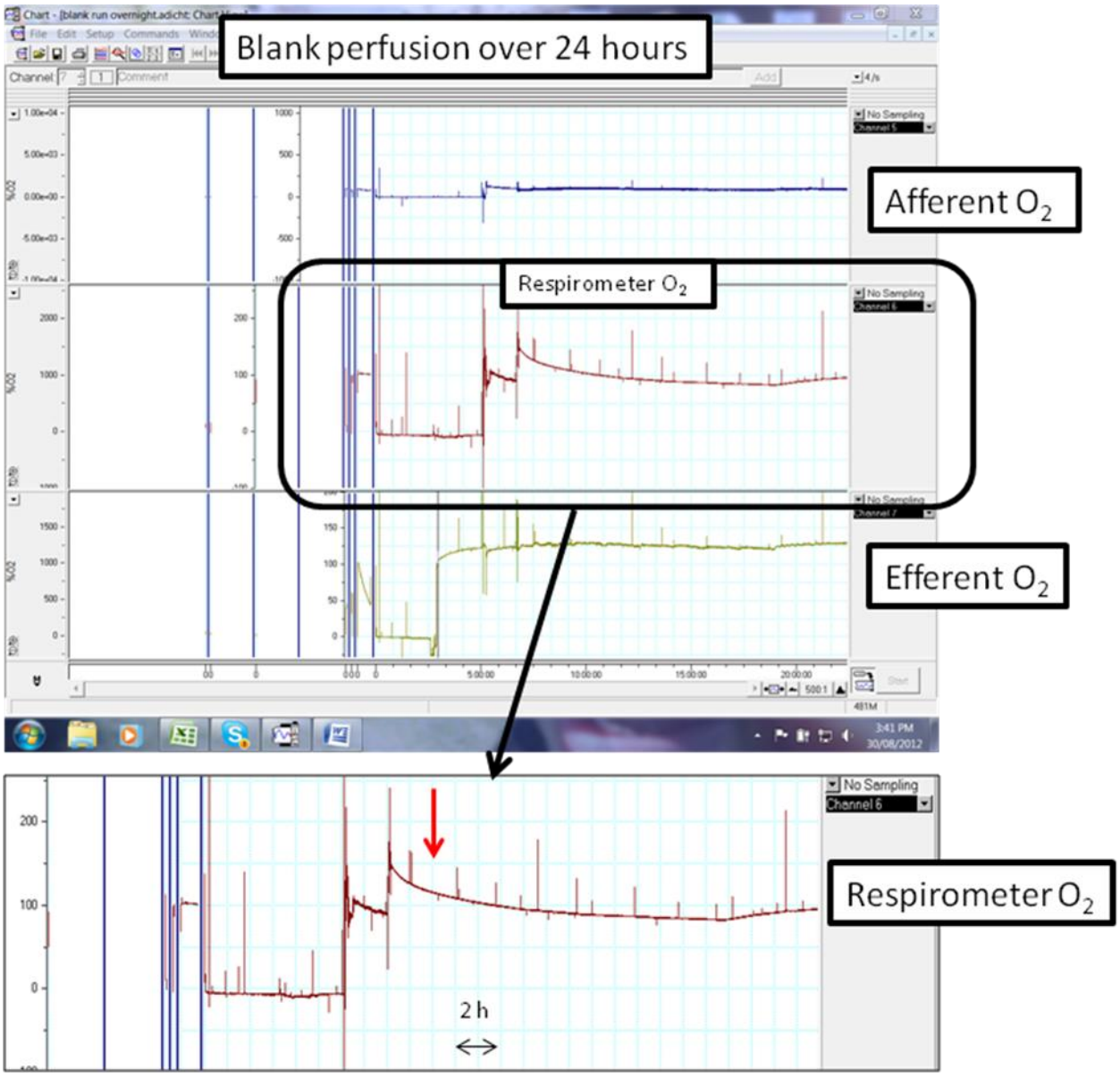

Figure 6. O_2 (%) recorded continuously over 20+ hours in afferent saline, seawater (gill chamber/respirometer) and efferent saline without the addition of a cannulated gill preparation. Note the red arrow indicates the end of calibration and start of blank chamber recording. Time scale in hours (h)

The results of this preliminary work clearly showed that a perfused gill preparation could be used for future respiration studies in Atlantic salmon. In agreement with the histological findings it is suggested the optimum time for experimentation using the current model will be within 3 hours of placement within the chamber. In addition it is recommended that larger fish are used in future experiments such as those outlined specifically for this project. The reasons for this recommendation are outlined below.

Cannulation of isolated arches from fish less than 700g was found to be considerably more difficult compared to larger animals due to the smaller

vessel diameter. The cannulation procedure took considerably more time and the preparations were prone to leakage that was observed at the cannulation sites. Investigation of the leaking preparations commonly found the cannula to be blocked which most likely resulted in significant back pressure. Leaking preparations had to be discarded. Modification of the cannula was also required due to small vessel size. The use of blunt end 23 G needles attached to saline filled PE50 tubing (cannula) was only found to be suitable for larger gills. For smaller fish the slight tapering of the PE50 tubing end was more successful. However, the use of blunt end 23 G needles would be a more favourable option as the tapered end tubing was easily occluded along with other vessels during cannula securement procedure involving silk suture.

The use of tissue adhesive (Vetbond) was insufficient for securing the cannula in place. The principal investigator noted that the cannula from these preparations was easily displaced either during cannulation of the other gill end or at the time of transfer from the surgical saline bath to the experimental chamber. Silk sutures tied around each cannula were therefore employed to further secure placement inside the vessel and also occlude the remaining (non cannulated) vessels. The use of silk sutures in conjunction with the tissue adhesive was the most successful method of cannulation.

The above results resulted in the final protocol (see below) that was used for subsequent experimentations.

Final protocol

Preparation of fish

1. Fish anesthetised with 20mg/l Aqui-S, weighed and then injected via the caudal vessels with heparinised (5000 U/Kg fish) Cortland's marine saline (see Table 2 for details)
2. Fish returned to anaesthetic bath for at least 5 min before increasing the anaesthetic dose of 40mg/l.
3. Following euthanasia a midline ventral incision is made to expose the heart and the tail removed.
4. The ventricle is pierced with a blunt 23G needle connected to a 30cm length of PE50 tubing. Perfusion with heparinised (10U/ml) saline is initiated and continued until the gills are cleared of blood.
5. The entire gill basket is removed and the first left gill arch excised and placed into aerated ice cold saline for cannulation.

Table 2. The chemical composition of Cortland's marine saline. Prior to use the saline was 0.2µm filtered.

Chemical	mM
NaCl	139
KCl	5.1
$CaCl_2$	1.1
$MgSO_4$	0.9
$NaHCO_3$	11.9
NaH_2PO_4	3.0
Glucose	5.6
Dextran	1%
pH 7.5	

Cannulation

6. The afferent (ventral) and efferent (dorsal) arteries are cannulated with blunt 23G needles connected to 15cm lengths of saline filled PE50 tubing
7. Each cannula is secured in place with silk suture.
8. Cyanoacrylate tissue adhesive (Vetbond 3M) is additionally used to occlude the remaining vessels at each end of the arch.

Experimental protocol

9. Placement of the preparation within the respiration chamber and attachment to the afferent and efferent electrodes (via saline filled lengths of PE50 tubing
10. Recording of O_2 measurements initiated
11. A 30 min acclimation period is observed prior to experimentation to the ensure the preparation is in a state of equilibrium
12. O_2 consumption will be recorded over a 3 h period
13. Following each perfusion the wet weight of the gill arch is recorded for calculation of oxygen consumption rate (MO_2) according to Lyndon (1994).

$$MO_2 = [(P_a - P_e) \cdot \alpha_{O_2} \cdot F] + [\Delta P_{chamber} \cdot \alpha_{O_2} \cdot V] / M$$

Where Pa and Pe are the afferent and efferent oxygen partial pressures of the perfusate (Torr), α_{O_2} is the oxygen capacity of water ($\mu mol.l^{-1}.Torr^{-1}$) according to Boutilier et al., (1984), F is flow rate ($l.h^{-1}$), $\Delta P_{chamber}$ is the change in oxygen partial pressure over time in the chamber ($Torr.h^{-1}$), V is the volume of the chamber (l) and M is the fresh weight of the gill (g).

14. After experimental procedures the preparation is placed into seawater Davidson's fixative for histological assessment of AGD severity and to ensure gill morphology and structure has been maintained.
15. The effects of AGD severity on oxygen consumption rate will be examined using correlation analysis.

In vitro assessment of gill function in AGD-affected fish

Oxygen consumption

The MO_2 calculated for fish in this study were similar to those reported for the perfused isolated gill of another salmonid species (Morgan and Iwama, 1999). Comparison of MO_2 in control and AGD-affected fish found a weak but significant relationship between affected filaments and MO_2 (*r=-0.506, p=0.045,* see Fig. 7). These results suggest that AGD does result in an impairment of oxygen uptake and consumption at the level of the gill. The negative relationship between the percentage of affected filaments and MO_2 suggested that this effect is more pronounced in gills that have advanced AGD severity.

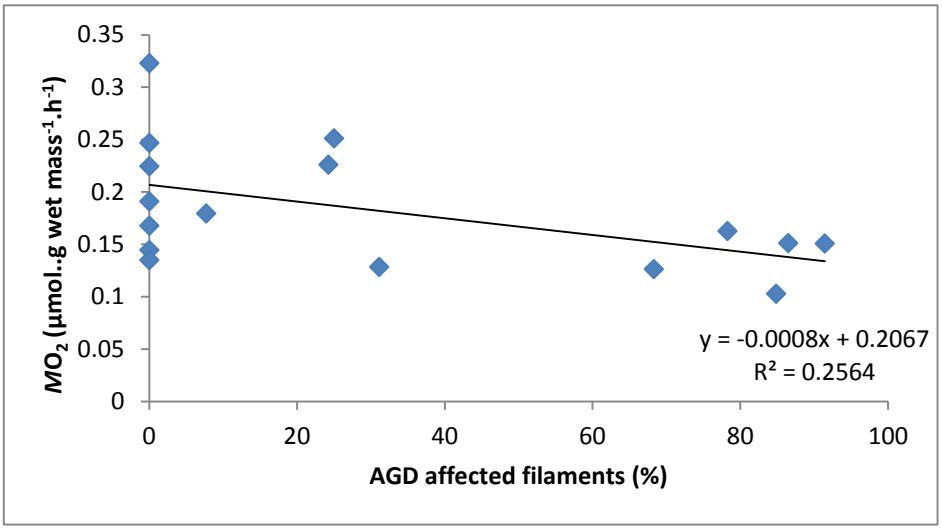

Figure 7. Relationship between MO_2 in AGD-affected filaments in Atlantic salmon, *Salmo salar.*

The addition of 0.5 mM ouabain to the saline perfusate resulted in a significantly smaller decrease in oxygen consumption compared to control (non AGD-affected) preparations (t=-3.66, df=3,p=0.035, mean ± SEM: 15.58 ± 2.67 and 28.81 ± 2.33 % for AGD and control fish respectively, see Fig. 8). The effect of the Na^+K^+ATPase blocker suggested that the oxygen costs associated with osmoregulation are lower in AGD-affected gills. Most likely this result is a consequence of the hyperplastic AGD lesions which effectively fill the non-respiratory areas of the gills where the majority of chloride cells reside (see Fig.9 for example).

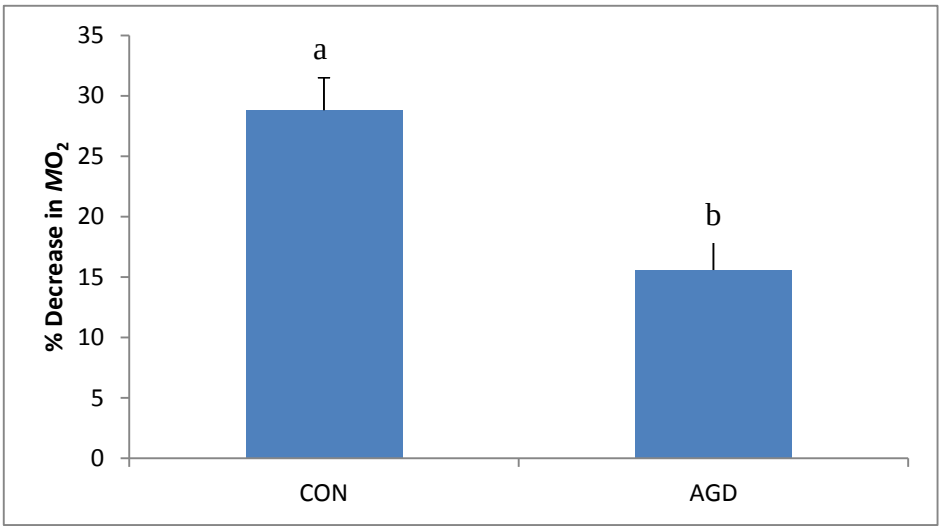

Figure 8. Mean ± SEM % decrease in MO_2 following addition of 0.5 mM ouabain to the saline perfusate. Different letters indicate a significant difference between AGD-affected perfused gill (AGD) and non-affected preparations (CON) means.

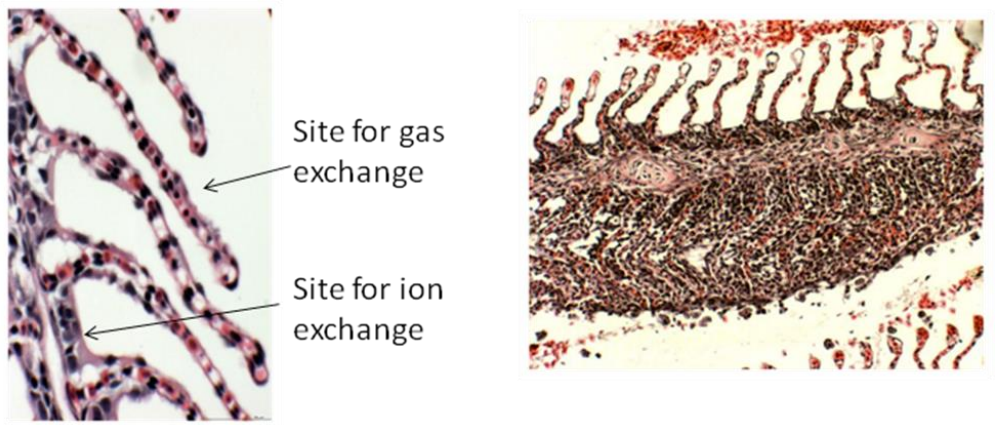

Figure 9. Typical AGD gill pathology showing loss of non-respiratory (ion exchange) areas of the gill.

Histology

Histological assessment of the AGD-affected gills initially used for this objective showed that although the fish were affected the level of AGD severity was low (up to 24.3% affected filaments). Subsequent cannulations of more heavily affected gills (up to 91.4% affected filaments) were included in this data set. Interestingly no amoebae were seen in association with the typical AGD lesions in histological sections taken of perfused preparations (Fig. 10). The absence of amoebae in the perfused sections was likely a consequence of prolonged exposure to the low salt saline solution following removal from the fish (entire branchial basket) and surgery (individual gill arches). Histopathology is considered the golden standard for AGD research and diagnosis, as it allows both host response (gill lesions) and the pathogen

(amoebae) to be visualised together. It is therefore suggested that an additional gill arch should be collected and fixed at time 0, post perfusion but prior to saline exposure, for definitive confirmation of AGD status.

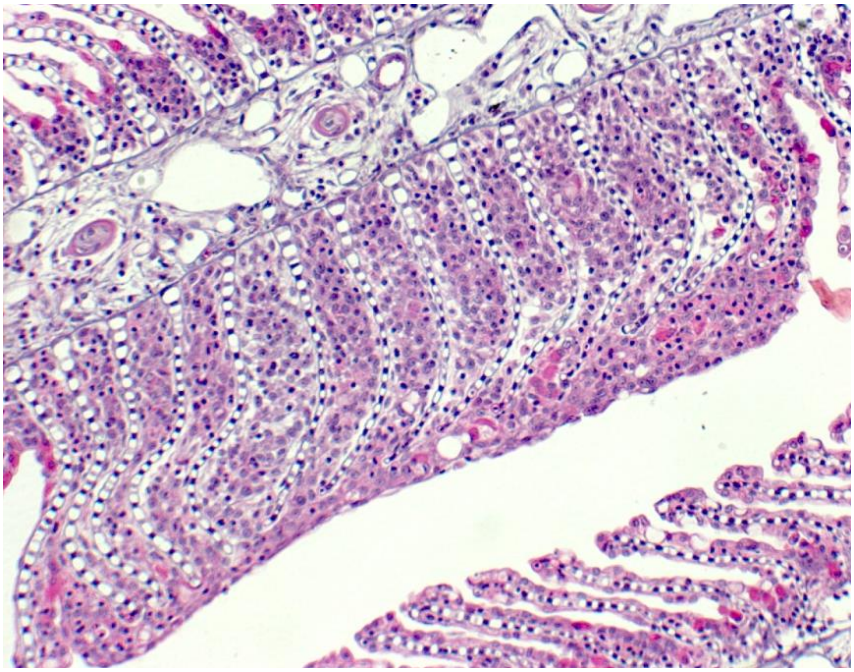

Figure 10. Histological section of a perfused gill fixed following experimentation showing absence of amoebae in association with the typical AGD lesion.

Investigation of altered gill blood flow patterns and branchial (gill) vascular resistance in AGD-affected fish

For investigation of branchial vascular resistance pressure transducers were placed on both the afferent and efferent side of the gill preparation. Pressure recordings were made for the period of experimentation for both AGD-affected and non-affected control gills. The increase in afferent pressure following attachment of the gill preparation to the perfusion loop was significantly higher in AGD-affected gills compared to control preparations (t=-3.967, df=6, p=0.007 mean ± SEM: 43.52 ± 1.74 and 61.52 ± 3.26 % for control and AGD fish respectively, see Fig. 11A). The increase in pressure seen in AGD-affected gills was also matched by a significant decrease in efferent pressure compared to controls (t=-2.762, df=6, p=0.033 mean ± SEM: 28.58 ± 2.30 and 54.72 ± 6.98 % for control and AGD fish respectively, see Fig. 11B). The difference in the change of both afferent and efferent pressures following attachment of the gill preparation in AGD-affected gills compared to controls suggests that AGD is associated with elevated branchial resistance. There were no appreciable differences in afferent or efferent pressure following administration of ouabain (data not shown).

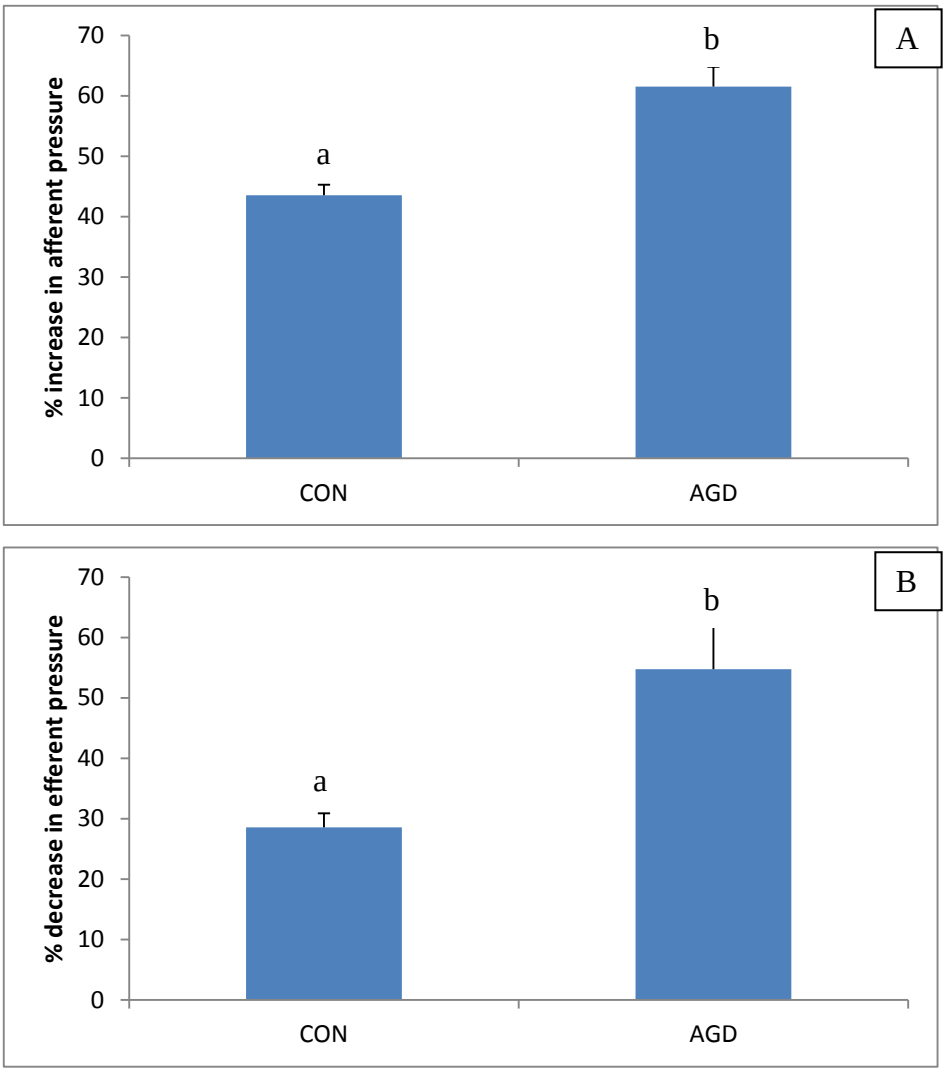

Figure 11. Mean ± SEM % change (increase or decrease) in afferent (A) and efferent (B) pressures following attachment of the gill preparation to the perfusion loop. Different letters indicate a significant difference between AGD-affected perfused gill (AGD) and non-affected preparations (CON) means.

Benefits and Adoptions

This project directly benefits the industry by providing an in vitro perfused gill model specifically for Atlantic salmon research. This model provides industry with a cost effective means for health and non-health related physiological research. The ability to manipulate the in vitro model to varying external (surrounding medium) and internal (perfusate) conditions provides a myriad of research applications. Additionally use of the model will additionally support a more ethically minded use of animals particularly in fish health research.

Further Development

One of primary constraint to intensification and continued growth of cultured fish industries is the occurrence infectious diseases. The economic effects of disease, as well as the growing concern and awareness for the welfare of cultured fish, have resulted in considerable research efforts directed into areas of fish health. Understanding the physiological changes that occur during infection provides greater insight into the mechanisms that cause physiological dysfunction and mortality. This knowledge can be used to highlight the best methods for disease mitigation and can provide alternative and novel avenues for research into treatments directed specifically to the host as opposed to the pathogen.

At present health related issues already represent a significant economic burden to industry and while the Tasmanian Salmon Growers Association (TSGA) has identified the area of fish health as a research priority, in vivo disease trials are inherently expensive. A more cost effective alternative to large scale in vivo research is use of an in vitro model as results from the model can be used to inform more comprehensive and expensive in vivo trials.

In light of industry interest, use of the model for host parasite interaction studies is needed. Further research aims specifically related to AGD could include:

1. Further development of the gill model for studies that would allow both attachment and detachment of amoebae to be visualised and confirmed.

2. Extension of the experimental viability of the model to allow development of AGD gill pathology to be observed in vitro.

Outcomes of this research would enable examination of the effects of potential AGD treatments, either waterborne, or expressed across the gill. The in vitro model offers a number of advantages including the ability to screen potential treatments prior to expensive and time consuming in vivo experiments. Additionally the model will highlight potential adverse treatment effects on gill function.

A non-surgical model for AGD in vivo research would complement and validate the results of the in vitro gill model. An in vivo whole animal respirometry approach will also have direct application for other health and non-health based physiological studies.

Planned Outcomes

This project was proactive and directly benefited salmon industry. The isolated perfused Atlantic salmon gill model has proved to be an effective in vitro model for AGD research. The primary end users and beneficiaries of this project are the TSGA, other researchers and the project investigators. While the benefits of this project are primarily non-pecuniary, the knowledge generated using this model could contribute to the elimination of AGD costs (approximately $15M). Successful mitigation of AGD will result in substantial reduction of health related costs both direct (labour, infrastructure, freshwater) and indirect (lost growth, mortality). Lower production costs will lead to more cost effective production and a more profitable and sustainable industry.

Dr Leef has successfully applied the model to AGD research and has provided 3 milestone reports detailing results to the FRDC. Additionally the co-investigator has had a visit with the TSGA chief executive Dr Adam Main and industry veterinarians Dr Steve Percival (late January 2013), Dr Dave Cockerill and Dr Dianne Morrison (both from Marine Harvest, late January 2013). Contact with Dr Alistair Brown and Dr Paul Hardy-Smith (industry veterinarians) was also made in early Feb 2013 where a presentation of the first experiment results was given at the Australian Society of Parasitology (ASP) funded workshop on fish parasites presented at the Australian Society of Parasitology: Parasitic Diseases in Fish Mariculture Workshop (Feb 2013). Results from this work were also presented at the FRDC Australasian Aquatic Animal Health Conference (July 2013). Greater dissemination of this research will be achieved by publication in peer reviewed international scientific journals. Publication of these results will directly benefit the project investigators as well as other researchers as the model could be then used for other health (non-AGD) related pathophysiological studies including investigation of bacterial disease, as well as non-health related physiological studies investigating the effects of climate change and elevated temperatures and salinities on oxygen consumption.

Conclusions

This study has provided proof of concept for the use of an isolated perfused gill model for AGD-affected Atlantic salmon. Manipulation of the model to varying external (surrounding medium) and internal (perfusate) conditions has allow direct examination of gill function in not only AGD-affected fish but also has direct application for other health and non-health based physiological studies. This model for instance could be used for other pathophysiological studies investigating the effects of other important Atlantic salmon pathogens. Various bacterial diseases including vibriosis, yersiniosis and flexibacteriosis are significant threats not only to the Tasmanian salmonid industry profitability but also the welfare of cultured fish species. In terms of non-health related physiological studies, the model could also be used to investigate the effects of climate change and elevated temperatures and salinities on oxygen consumption in this important fish species.

The in vitro perfused gill preparation offers a novel approach to AGD research and it has provided the first in vitro assessment of gill function in AGD-affected fish. The results of this study indicate that AGD does result in an impairment of oxygen uptake and consumption at the level of the gill. The negative relationship between the percentage of affected filaments and MO_2 suggest that this effect is more pronounced in gills that have advanced AGD severity. The effect of the Na^+K^+ATPase blocker ouabain additionally suggests that the oxygen costs associated with osmoregulation are lower in AGD-affected gills. This result most likely is a consequence of the hyperplastic AGD lesions which effectively fill the non-respiratory areas of the gills where the majority of chloride cells reside.

Results from this project have also provided insight into the branchial resistance and potentially blood distribution and flow within the gills. The difference in the change of both afferent and efferent pressures following attachment of the gill preparation in AGD-affected gills compared to controls. This suggests that AGD is associated with elevated branchial resistance.

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Appendix 1

INTELLECTUAL PROPERTY

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Appendix 2

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