Final Report

Atlantic Salmon Subprogram: Oxygen regulation in Tasmanian Atlantic salmon

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OBJECTIVES:

1. Determine the proportion of selected families that are oxygen regulators during seawater transfer
2. Provide an industry definition of a hypoxic event and link to management practice
3. Determine the physiological effects of a routine hypoxic event and if they differ among families
4. Determine if the ability to regulate is affected by swimming and if it differs among families

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE (boxed)

This project has confirmed that oxygen regulators do occur within the Tasmanian population of Atlantic salmon.

The proportion of oxygen regulators differs among families: differences occur among female salmon in freshwater and males in seawater, indicating that there may be a genetic component to oxygen regulation.

Oxygen regulators have a lower metabolic rate than oxygen conformers.

Bases on physiological changes a working definition of a hypoxic event in both freshwater and seawater was presented.

Oxygen regulation occurs in both static and swimming conditions.

This project primarily addresses physiological "robustness" in Atlantic salmon. Critical issues for the Tasmanian salmon industry include understanding the physiological response of salmon to hypoxia due to decreased dissolved oxygen (DO) and increases in other dissolved metabolic wastes. The Tasmanian salmon industry has a selective breeding program and robustness/resilience is one of the main characteristics the industry is interested in developing further. Meanwhile, the Industry is investing heavily in order to manage the DO environment experienced by its fish. For example, SALTAS spends more than $500,000 annually (i.e., 10% of its operational budget) on oxygen to help combat the high temperature/low DO issues.

Contrary to conventional wisdom, some of our preliminary research has shown that some salmon appear able to regulate their metabolism in response to increasing hypoxia. This unexpected characteristic in Atlantic salmon has the potential to contribute significantly to survival during critical environmental events (e.g. hypoxia) experienced during both the freshwater and seawater phases of culture. The current research project provides baseline data on the proportion of salmon from different and unrelated families that regulate and then investigates the effect of the transfer to seawater, fish size, stage, and environmental history on this ability. Ultimately the research will contribute to determining which phenotypic characteristics have a genetic basis and might therefore be exploited in selecting for a more robust salmon.

The current project has confirmed that oxygen regulation does occur in the Tasmanian population of Atlantic salmon. A number of key findings regarding oxygen regulation have resulted from this project and include differences among pedigreed families, differences between the metabolic rates of oxygen regulators and conformers, differences between male and female fish and that oxygen regulation occurs under both static and flow conditions.

Differences among families were found in the ability to oxygen regulate. Between 30% and 64% of fish in the initial three families tested were able to oxygen regulate. Fish which have the ability to oxygen regulate have approximately 40% lower metabolic rate than those individual fish which are oxygen conformers. These results are interesting because reduced metabolic rates may relate to the overall robustness of the fish but more importantly to the overall efficiency of the individual

fish. Distinct differences between male and female fish became apparent during static respirometry experiments with female fish having a higher metabolic rate, higher critical oxygen thresholds, higher stress and lactate levels than male fish from the same families. This has potential significance for industry owing to the high reliance on all female populations and warrants further investigation.

When large numbers of individuals were measured over a dissolved oxygen gradient (100 to 18% oxygen saturation), a number of distinct patterns emerged in both freshwater and seawater. In freshwater, the first parameter to be affected by the decrease in DO concentration was haematocrit level which increased until DO reached 5.37 mg O_2/L where it then stabilised, the same pattern was observed in seawater however the plateau did not occur until DO reached 3.69 mg O_2/L. This indicated that during mild hypoxia fish increased the proportion of red blood cells (RBC) in their blood, presumably to cope with the decreasing oxygen levels. Cortisol remained constant over the DO gradient until the DO concentration reached 3.61 mg O_2/L (~35% saturation) in freshwater and 4.27 mg O_2/L (~50% saturation) in seawater. Once these critical thresholds were crossed, the stress levels increased by an order of magnitude.

This research was primarily designed to gather baseline data on a wide range of parameters relating to decreasing dissolved oxygen. Further research is needed to properly investigate the extent of the family differences by examining greater numbers of fish from a greater number of families as well as determining differences between both males and females from each pedigreed family. It will be critical to examine differences between individuals at high (i.e. summer) temperatures because hypoxia increases as temperature increases. To determine if the ability to oxygen regulate is genetic or an adaptive response, additional research is needed to establish whether preconditioning to hypoxia (or any stressor) increases tolerance and/or decreases time to recovery.

KEYWORDS: Atlantic salmon, aquaculture, oxygen consumption, oxygen regulation

Acknowledgements

This study formed part of salmon industry research, and was supported by the Fisheries Research and Development Corporation. We would like to thank the salmon industry for their collaboration and support. We would also like to thank Brad Evans with support through the selective breeding program and Mark Adams for technical assistance.

We would also like to thank Ryan Wilkinson, Edward Barnes and Daniel Poutney for their help with sampling. Steve Percival for his help sampling fish at the Huon Aquaculture Company. Annabel Tyson for managing the accounts associated with this project.

Background

This project primarily addresses physiological "robustness" in Atlantic salmon. Critical issues for the Tasmanian salmon industry include understanding the physiological response of salmon to decreased dissolved oxygen (DO) and increases in other dissolved metabolic wastes. An important practical outcome involves identification of individual salmon that are more robust and better able to tolerate poor conditions. The Tasmanian salmon industry has a selective breeding program and robustness/resilience is one of the main characteristics the industry is interested developing further. Meanwhile, the Industry is investing heavily in order to manage the DO environment experienced by its fish. For example, SALTAS spends more than $500,000 annually (i.e., 10% of its operational budget) on oxygen to help combat the high temperature/low DO issues.

The research was initially proposed by Dr. King (Salmon Enterprises of Tasmania Pty. Ltd. - SALTAS) to resolve an unconfirmed observation of metabolic regulation by salmon. Funded by a DAFF award, Dr. Katersky (UTAS) confirmed that oxygen regulation does occur (Appendix 3). The results show that oxygen regulation occurs in up to 75% of Tasmanian Atlantic salmon at a range of temperatures (14, 18 and 22C) in freshwater. From these results a project was originally designed to examine the effect of high temperature on oxygen regulation because the salmon industry can

experience impaired growth performance and high levels of mortality associated with elevated summer water temperatures which simultaneously lower DO and increase the salmon's oxygen demand. Water temperatures above an optimal level of c. 16°C are common and can rise above 22°C in extreme conditions. At 22°C, compared with the optimum temperature, the DO concentration is reduced by 15% at saturation. Elevated temperatures already approach the thermal tolerance limits for the farmed Atlantic salmon population and the Tasmanian salmon industry is well aware that this problem will only worsen because global temperatures are predicted to increase by at least 2°C by 2070 and even greater increases are expected in the Tasman Sea (Hobday, et al., 2006). Temperature is not the primary concern in the current application but through consultation with industry it may become a factor in future research. While working with industry to refine this project, a number of meetings were held. On August 26th, 2009 it became clear that the marine sector of the Tasmanian Salmon industry was interested in a pilot study to determine if the ability to oxygen regulate was present in the seawater phase. On October 1, 2009, the importance of determining whether variation in oxygen regulation occurs among families (i.e. there is a genetic link to this ability) was identified.

This project has been refined based on the advice of industry to address both of these issues.

Need

Our recent research has confirmed that, contrary to conventional wisdom, some salmon appear able to regulate their metabolism in response to increasing hypoxia. This unexpected characteristic has the potential to contribute significantly to survival during critical environmental events (e.g. hypoxia) experienced during culture. Furthermore, the observation of individual variation raises the possibility of being able to select for the characteristic. However, before this can be done key questions around the phenotypic expression of regulation need to be addressed: is the ability to regulate innate or does exposure to one or more hypoxic episodes cause salmon to express the ability to regulate? This research provides baseline data on the proportion of salmon from different and unrelated families (family information and fish

were provided by SALTAS) that regulate and then investigates the effect of the transfer to seawater, fish size, stage, and environmental history on this ability. Ultimately the research will contribute to determining which phenotypic characteristics have a genetic basis and would therefore be valuable in selecting for a more robust salmon.

This research is strongly aligned with the Fisheries Research and Development Corporation's R&D Plan for 2005-2010 and the strategic challenge of Natural Resource Sustainability. It directly addresses the challenge for the Tasmanian salmon industry to manage the effects of a changing climate through understanding how environmental variables affect the physiology of salmon and what level of phenotypic variation exists in the population. In the "Tasmanian Fisheries and Aquaculture Research and Development Strategic Plan 2005-2008" the relevant research and development priorities are "climate variability" and "genetic improvement". This research directly addresses climate variability with the affects of hypoxia on salmon physiology and genetic improvement by testing fish from different and unrelated families to determine if oxygen regulation is an innate or learned response to a variable environment.

Objectives

1. Determine the proportion of selected families that are oxygen regulators during seawater transfer
2. Provide an industry definition of a hypoxic event and link to management practice
3. Determine the physiological effects of a routine hypoxic event and if they differ among families
4. Determine if the ability to regulate is affected by swimming and if it differs among families

Methods

Experimental Fish

All experimental fish were sourced from Salmon Enterprises of Tasmania Pty Ltd (SALTAS) and were held at the University of Tasmania's, National Centre for Marine Conservation and Resource Sustainability's (NCMCRS) aquaculture facility in Launceston, Tasmania. Fish were held under constant conditions (11.5 ± 0.1°C and a 10:14 light:dark photoperiod) in 2300-L recirculation systems consisting of one 2000-L tank with a 300-L sump which contained a biofilter. Water quality was maintained within the limits for Atlantic salmon and water exchanges were made as necessary. Fish were fed to satiation daily with a commercial diet (Spectra SS) provided by Skretting Australia (Cambridge, Tasmania, Australia).

Progressive Hypoxia Experiments

The first aim of these experiments was to determine the proportion of each separate family which were oxygen regulators in order to determine if there was a genetic component to regulation. Following this, it was of great interest to determine if the fish's ability, or lack of it, to regulate their metabolic rate continued through seawater transfer.

Freshwater (Experiment 2)

This experiment was conducted from 6 July, 2010 – 11 August, 2010. Individual Atlantic salmon (n=35) from the 08YC (both males and females from three families) were placed into 350-L static respirometer chambers. There were four individual chambers in total and each chamber was fitted with a HACH IntelliCAL Luminescent dissolved oxygen probe (HACH Company, Loveland, Colorado, USA) which was calibrated with air-saturated water and a HACH IntelliCAL pH probe (HACH Company, Loveland, Colorado, USA). Once fish were placed in the chamber, a plexi-glass dome lid was sealed and tested to ensure a water-tight fit (Fig. 1). Once sealed, the water flow was adjusted to 9.5 $L \cdot min^{-1}$ and the fish acclimated overnight (for a minimum of 12 h). The following morning, any remaining air bubbles were removed and the water flow stopped. Oxygen and pH measurements were automatically recorded every five minutes until the fish lost its equilibrium. At this time the fish was removed from the respirometer chamber and euthanized by

anaesthetic overdose (Aqui-S 50 mg/L). An arterial blood sample was immediately taken prior to the fish being weighed and fork length measured. Immediately following this, caudal blood and tissue samples were then taken for haematology, cortisol, lactate and glycogen measurements. During each run, one chamber was left empty to serve as a control and the oxygen consumption of the control chamber was subtracted from the oxygen consumption of the test chamber and metabolic rate was calculated as:

$$VO_2 = ((Co_{2A} - Co_{2B}) \cdot T^{-1}) \cdot V / W \quad (1)$$

Where VO_2 is the metabolic rate (mg/kg/h), Co_{2A} is the oxygen concentration (mg/L) at the start of the measurement period, Co_{2B} is the oxygen concentration (mg/L) at the end of the measurement period, T is the time between the measurements (h), V is the volume of the respirometer chamber (350L) and W is the whole fish weight (kg) (Cech Jr., 1990). VO_2 was calculated for all fish. For fish that were classified as oxygen regulators a mean VO_{2PL} was calculated from normoxic levels down to the critical oxygen threshold (P_{crit}). For Fish that were classified as oxygen conformers a mean VO_2 was calculated from metabolic rates determined at 80-100% water saturation levels.

Seawater (Experiment 5)

This experiment was conducted from 14 April, 2011 – 5 June, 2011. Individual Atlantic salmon (n=14) from the 09YC (males from three families) were placed into 350-L static respirometer chambers. These fish were provided by Salmon Enterprises of Tasmania Pty. Ltd. (SALTAS) on April, 12th, 2011 and were brought to the University of Tasmania's NCMCRS aquaculture facility in Launceston and were held at 12°C, 10:14 light:dark photoperiod in freshwater until the start of the experiments. These fish had already undergone conditioning for the spawning season and therefore the experiments were conducted on starved fish. All fish that were used in this experiment were ripe males with the exception of one fish which was female (which was subsequently excluded from the analysis). There were two phases in this experiment. The first was to examine whether fish were oxygen regulators or conformers in freshwater (50% of the fish were tested for this), then the fish were slowly acclimated to seawater (over a three week period) and held in

seawater (>30‰) for an additional four weeks before testing all fish to determine if the ability to regulate or conform is maintained during seawater transfer and across families. Progressive hypoxia experiments were run according to the above methods for experiment #2. In the freshwater phase, it should be noted that progressive hypoxia experiments were terminated prior to the fish reaching the critical oxygen threshold (P_{crit}) this was done in order to ensure the fish were not exposed to oxygen concentrations which would have induced stress (based on experiment #3 in freshwater).

Defining Hypoxia Experiments

The aim of these experiments was to determine the oxygen concentration at which physiological changes occurred in both fresh and seawater.

Freshwater (Experiment #3)

This experiment was performed over a period of 22 h on June 16th -17th, 2010 at the University of Tasmania's NCMCRS aquaculture facility in Launceston, Tasmania. Fish (400) were transferred from the SALTAS hatchery on April 8th, 2010 and held in a 2300-L recirculation system (as described above) until the start of the experiment. Fish were fed to satiation daily and held at a constant temperature (13.5 ± 0.56°C) during this period. One week prior to the start of the experiment, 100 fish were transferred to an identical system under the identical conditions and allowed to acclimate to the new tank for one week. These fish were sampled at four times during the experiment to serve as a control group for haematology, cortisol and metabolic measurements.

At the start of the experiments, an initial group of fish (n=10) was sampled from the experimental tank. Each fish was removed from the tank, quickly killed with a sharp blow to the head and had an arterial blood sample (25G needles, pre-heparinised) taken in order to determine the pH and oxygen saturation in the blood. Another blood sample was taken from the caudal vein and separated into two aliquots. One aliquot was kept as whole blood for analysis of haematocrit (HCT) and haemoglobin (Hb) and the other was spun down for plasma collection for cortisol and lactate analysis. The fish were then weighed (g) and fork length measured (mm) before

white muscle, liver and brain tissues were removed and immediately frozen in liquid nitrogen for later analysis of glycogen.

After the initial samples were taken, the water and aeration to the tank was shut off and the oxygen content of the water was allowed to decrease as the fish consumed the oxygen. Fish (n=6) were sampled as the dissolved oxygen content decreased at 5% intervals (ie. 100, 95, 90, 85, 80% saturation etc.) until loss of equilibrium (LOE) occurred. An additional six fish were sampled immediately on LOE.

Groups of fish (n=5) were sampled from the control tank four times over the course of each experiment. Sampling procedure was identical to that previously described with the exception of the arterial blood gas sample which was omitted from the control tank.

Seawater (Experiment #6)

This experiment was performed on 11th -12th August, 2010 at the Aquaculture facility at the University of Tasmania in Launceston, Tasmania. Smolts were transferred from the SALTAS hatchery on July 8th, 2010 and placed directly into 20‰ sea water. Fish were acclimated to >30‰ over one week where they were held at a constant salinity for 4 weeks prior to the experiment. Fish were fed to satiation daily and held at a constant temperature (11.6 ± 0.42°C) during this period. One week prior to the start of the experiment, 100 fish were transferred to an identical system under identical conditions and allowed to acclimate to the new tank for one week. The remainder of the experiment employed methods were identical to those utilised in Experiment #3.

Recovery from a hypoxic event (Experiment # 4)

The aim of this experiment was to determine the effects of a hypoxic event on individual fish and if it differed between individuals from different families, thereby identifying a potential genetic component to the ability to cope with hypoxia. This experiment was conducted from 9-19^{th,} May 2011 with fish from the 09YC. These fish were provided by Salmon Enterprises of Tasmania Pty. Ltd. (SALTAS) on April, 12th, 2011 and were brought to the University of Tasmania's Aquaculture Centre in Launceston. These fish had already undergone conditioning for the spawning

season and therefore the experiments were on starved fish. All fish that were used in this experiment were ripe males. Fish were acclimated to sea water (> 30‰) over a three week period. Fish from two pedigreed families (5 fish per family) were used plus an additional four fish from random families. The total number of fish examined was 14 fish.

Individual fish were transferred into one of four, 350L static respirometers and allowed to acclimate overnight (> 12 h) in fully oxygenated flowing water. At the start of the experiment, the flow was turned off, all air was removed from each respirometer and a HACH LDO IntelliCal DO probe was fitted into the respirometer which was then completely sealed. The experiment lasted until fish passed the critical oxygen threshold (as determined from experiment #2, mean COT for all families was 3.09 mg/L) at 3 mg/L once the fish passed this point the flow to the tank was reinstated and the fish allowed to recover from the hypoxia exposure. At 11h after the flow was reinstated, a 1h metabolic rate check was performed in order to determine if the fish's metabolic rate had returned to previous levels. At 12h, the fish was euthanized and blood and tissue samples were taken for haematology, cortisol, lactate and glycogen measurements. A recovery time of 12h was selected based on the literature which indicated that differences would still be apparent at 12h following a severely hypoxic event and that by 24h recovery from hypoxia should have occurred.

Swimming Respirometry (Experiment #7)

The aim of this experiment was to determine if the fish were able to oxygen regulate under normal flow conditions experienced on farm. This experiment was similar to the progressive hypoxia experiments above with the exception that it was run in a swimming respirometer (170L) which had a constant flow rate of 35.2 cm/s. This experiment was run from 31 May – 2 July 2011 with individual fish (167.3 ± 33.5 g) from a general population. These fish were originally provided by Salmon Enterprises of Tasmania Pty. Ltd. (SALTAS) and were held at the University of Tasmania's Aquaculture Centre in Launceston in seawater at 12°C, 10:14 light:dark photoperiod until the start of the experiments.

The swimming respirometer (Fig. 2) is a 170L respirometer with an 80L chamber to house the fish. The respirometer is attached to a 3-phase CMG 5.5 kW pump and controlled by a TECO 7300CV inverter (TECO Electric and Machinery Co, Ltd., Taiwan). The water flow was calibrated using a General Oceanics flow meter (Model #2035 MKIV) to a speed of 35.2 cm/s with the aim of the swim speed being between 1 - 2 body lengths/second (BL/s) depending on the size of the fish (Piggott, 2008). A HACH LDO IntelliCal DO probe was fitted into the respirometer and oxygen measurements were taken every 5 minutes.

Individual fish were placed in the swimming respirometer and allowed to acclimate to the swimming chamber overnight. Fish were also "trained" in the respirometer by switching the flow on for approximately two hours to ensure that the fish was acclimated to the set flow rate. After the acclimation period, the flow was turned on and any remaining air in the respirometer was removed. Fish were then allowed to consume the available oxygen for 24h. At this point, the flow was stopped and the fish removed from the respirometer, blood samples for haematology, cortisol and lactate were taken and the weight and length of fish measured. Ten fish were measured in the swimming respirometer with an average swimming speed of 1.33 ± 0.14 BL/s.

Blood Gas and Haematology

Arterial blood samples (~500 µL) were collected from the dorsal aorta using a 25G pre-heparinised 1mL syringe (Cech Jr., 1990). Blood gas was measured using flow-thru pH and O_2 microelectrodes (Microelectrodes Inc., Bedford, New Hampshire, USA) attached to a PowerLab® data acquisition system (AD Instruments, Castle Hill, Australia) which was interfaced to a laptop computer. The pH probe was calibrated using standard pH buffers, the O_2 electrode was calibrated by using 100% air saturated water and a 2% $NaSO_2$ (zero) solution of water (Leef, et al., 2007).

Haematocrit (HCT) was immediately measured from caudal blood in duplicate using two heparinised 20 µl microhaematocrit tubes placed in a microhaematocrit centrifuge (Premiere model XC-3000, Manassas, Virginia, USA) and spun at 12,000

rpm for 5 min. HCT for was then read directly as a percentage using a micro-haematocrit capillary tube reader (Hawksley and Sons, Ltd, Sussex, UK).

A whole blood aliquot (at least 500 µl) was taken from the caudal vein sample via a pre-heparinised syringe and frozen for later analysis. To measure whole blood Hb concentration a 20 µl whole blood sample was added to 980 µl of Drabkin's reagent (Sigma-Aldrich Chemical Company, Castle Hill, NSW, Australia). Hb concentration was determined spectrophotometrically at 540 nm and quantified against a standard curve of known concentrations using spray dried Hb (Sigma). Mean corpuscular haemoglobin concentration (MCHC) was calculated according to the following equation:

$$MCHC = Hb / HCT \quad (2)$$

where, Hb is the haemoglobin concentration (g/dL) and HCT is the haematocrit value (%).

Cortisol, Lactate and Glycogen

Plasma cortisol was analysed using an enzyme immunoassay cortisol test kit (Cortosolo EIA Well #KS18EW, Radim Diagnostics, Pomezia, Italy). Test kits were validated through serial dilution of samples and repeating samples using a tritiated RIA as described by Pankhurst and Carragher (1992).

Plasma was analysed for lactate using an EnzyChrom^{TM} L-Lactate Assay Kit (ECLC-100, BioAssay Systems, Hayward, CA, USA). Lactate concentrations were determined spectrophotometrically at 565 nm and quantified against a known standard curve.

White muscle, liver and brain glycogen levels were determined using an EnzyChrom^{TM} Glycogen Assay Kit (EGCN-100, BioAssay Systems, Hayward, CA, USA). Samples were prepared according to the established methods of Keppler and Decker (1974) prior to use in the assay kit.

Statistical Analysis

All data are presented as mean ± standard deviation. For progressive hypoxia, recovery and swimming experiments (experiments #2, 4, 5 and 7), each individual fish had a piece-wise regression conducted on the raw dissolved oxygen (DO) measurement data (every five minutes) to determine whether the fish was a regulator or a conformer and to determine the critical oxygen threshold (P_{crit}). A fish was determined to be an oxygen regulator according to the methods of Barnes et al., (2011). Briefly, this included meeting a number of criteria: 1) the piece-wise regression was significant overall ($p<0.05$); 2) a P_{crit} could be identified; 3) the rate of oxygen consumption below the P_{crit} decreased linearly; 4) there was no significant relationship between oxygen concentration and the rate of oxygen consumption above the P_{crit} , therefore there was a plateau in the metabolic rate; 5) the plateau lasted for a minimum of three hours. ANOVA was used to determine whether differences occurred between the mean metabolic rate, Pcrit and physiological parameters and individual families where applicable. T-test was used to determine differences between regulators and conformers as well as males and females (experiment #2). For defining hypoxia experiments (experiments # 3 and 6) regression analysis was used to determine the relationship between water dissolved oxygen concentration and each measured parameter. If this was significant, a break point analysis was performed to determine the point along the oxygen gradient where a physiological change occurred. All differences were considered significant at $p\leq0.05$.

Figure 1. Static respirometer system

Figure 2. Swimming respirometer

Results/Discussion

1. Determine the proportion of selected families that are oxygen regulators during seawater transfer

Freshwater (Experiment #2)

Three pedigreed families (males and females) were examined from the 2008 year-class of the SALTAS selective breeding program. Families were selected based on their ranking within the selective breeding program and reflected a range of overall performance levels as well as those families where the largest numbers of fish were available. Fish were maintained in freshwater for this initial experiment. The authors had previously conducted experiments in freshwater on small fish (Barnes, et al., 2011), the present experiment aimed to address an industry concern that the ability to oxygen regulate may be size dependant and also to confirm the original finding that Tasmanian Altantic salmon actually oxygen regulate. It was therefore important to obtain data from larger fish to confirm our previous result as well as determine if there was a genetic component to oxygen regulation by comparing individuals from different families. To build on our previous results it was also important to understand what physiological and genetic differences occurred in freshwater prior to determining if the ability to oxygen regulate occurred after seawater transfer.

From each family, between 60 and 80% of males were oxygen regulators. The occurrence of oxygen regulation was consistent with previous observations (Barnes, et al., 2011). The metabolic rates for each family were not significantly different from each other ($p=0.701$, $df=12$, $F=0.368$) and the average was 100.01 ± 15.94 mg/kg/ h (Fig. 3). The metabolic rates for starved males in the current study were consistent with literature values for adult fish (Enders and Scruton, 2005). The critical oxygen threshold (P_{crit}) is the concentration of oxygen where oxygen regulators are no longer able to regulate and become oxygen conformers. This is a critical piece of information because it provides industry with an indication of where critical physiological processes are occurring and also can be an indicator of hypoxia tolerance (Chapman, et al., 2002). Individuals with a lower P_{crit} would be considered more hypoxia tolerant (Routley, et al., 2002). In the three families examined in the

current study, there was no significant difference in P_{crit} (p=0.606, df=12, F= 0.527) which averaged 2.99 ±1.18 mg O_2/L. Individual values ranged from 1.97 to 5.50 mg O_2/L for males from across the three families (Fig. 3), indicating that there is considerable individual variation and that certain individuals exhibited much greater hypoxia tolerance than others.

Differences did occur among families for haematocrit (HCT) values with family #2 having a significantly higher HCT than family #1, however family #3 was not different from either of the other families (p=0.05, F= 3.95, df=2; Table 1). These differences in HCT may indicate important haematological changes which occur once the P_{crit} has been reached (Farrell and Richards, 2009). With a greater proportion of red blood cells present (as with family #2), the fish would have the potential to increase the oxygen carrying capacity of the blood. Differences also occurred among the families for liver glycogen (p=0.04, F=5.30, df=2) with family #2 having significantly lower levels than the remaining families (Table 1). Liver glycogen becomes a more important fuel source when fish are held at low oxygen concentrations for prolonged periods of time (Richards, 2009). Fish which are considered hypoxia tolerant have higher glycogen levels especially in the liver and are able to mobilise this glycogen into the bloodstream and then to other tissues which are able to cope with lower levels of oxygen for longer periods (Richards, 2009). No other significant differences occurred for haemoglobin, MCHC, lactate, plasma cortisol, white muscle or brain glycogen for males from the three families examined (Table 1).

It has been shown previously that males and female Atlantic salmon have significantly different metabolic rates (Kazakov and Khalyapina, 1981). Fifteen females from the same three families were examined in the present research. The female results indicate that between 0 and 50% of the fish were oxygen regulators, no females in family 1 were oxygen regulators while 50% were oxygen regulators from families 2 and 3. For female oxygen regulators from the two families which had oxygen regulators (Fig. 4) there were no differences between the routine metabolic rates (t=1.52, df=3, p=0.37) or P_{crit} (t=1.83, df=3, p=0.26) and were on average 126.74 ± 19.89 mg O_2/kg/h and 4.14 ± 0.77mg O_2/L, respectively. For females,

mean corpuscular haemoglobin concentration was significantly different among families (p=0.04, F=5.29, df=2), however with the limited samples from family #2, it was not possible to clearly determine which families were different (Table 1). No other significant differences occurred among families for HCT, Hb, plasma lactate, plasma cortisol, white muscle, liver or brain glycogen (Table 1).

Significant differences did occur between males and females. Overall males had a significantly lower metabolic rate (100.01 ± 15.94 mg O_2/kg/h) than females (126.74 ± 19.89 mg O_2/kg/ h; t=-2.69, df= 16, p = 0.035; Fig. 5). Previous research which examined male and female adult Altantic salmon separately indicated that wild males have a higher metabolic rate than females (Kazakov and Khalyapina, 1981), however in this study we clearly showed the reverse. Differences were also apparent in the P_{crit} with males (2.99 ± 1.18 mg O_2/L) having a significantly lower P_{crit} than females (4.14 ± 0.77 mg O_2/L ; t= -2.69, df=16, p=0.034, Fig. 5), indicating that females may be more susceptible to hypoxia than males. Coupled with the difference in the occurrence of regulators between families this could be of critical significance for industry as the reliance on an all female stock is high.

There were differences in HCT between male and females. Males had a significantly higher HCT (55.61 ± 6.46%) than females (48.42 ± 9.75%; t=2.27, df=25, p=0.032; Fig. 6). This indicates that the males have a higher percentage of red blood cells in their blood (Table 2). Differences between males and females were also apparent for plasma lactate (t=-2.42, df=27, p=0.023; Fig. 7) and cortisol (t=-3.05, df=19, p=0.007; Fig. 8). Females reached cortisol and lactate levels which were 33% higher than males. While all fish were stressed from exposure to severe hypoxia at the time of sampling (Trenzado, et al., 2008), the level of stress and lactate accumulation for female fish indicates the female fish have lower tolerance to hypoxia and exhibit a greater stress and biochemical response to it. No differences occurred between males and females for haemoglobin, MCHC or tissue glycogen (Table 2).

When the male and female datasets were combined, overall the proportion of fish that oxygen regulated varied between 30.0 – 64.3% for the three families, however there were still no difference among the three families for metabolic rate (p=0.33,

df=2, F=1.193) or the critical oxygen threshold (p=.245, df=2, F=1.546). It should be noted that this conclusion was based on relatively low numbers of fish (only 35 fish spread among the three families were tested).

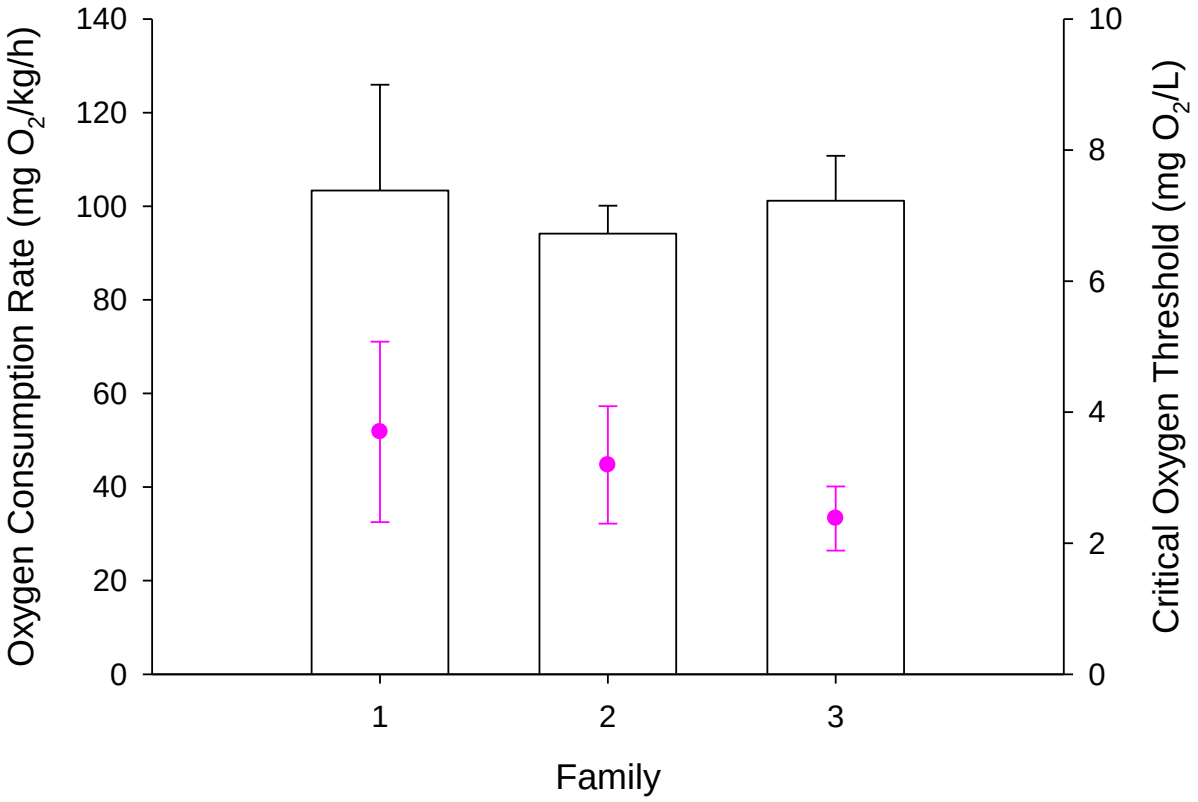

Figure 3. The mean metabolic rates (mg O_2/kg/h, bars) for males from the three families tested in freshwater (p=0.701, df=12, F=0.368) as well as the mean critical oxygen threshold (mg O_2/L) of males (●) for each family (p=0.606, df=12, F= 0.527).

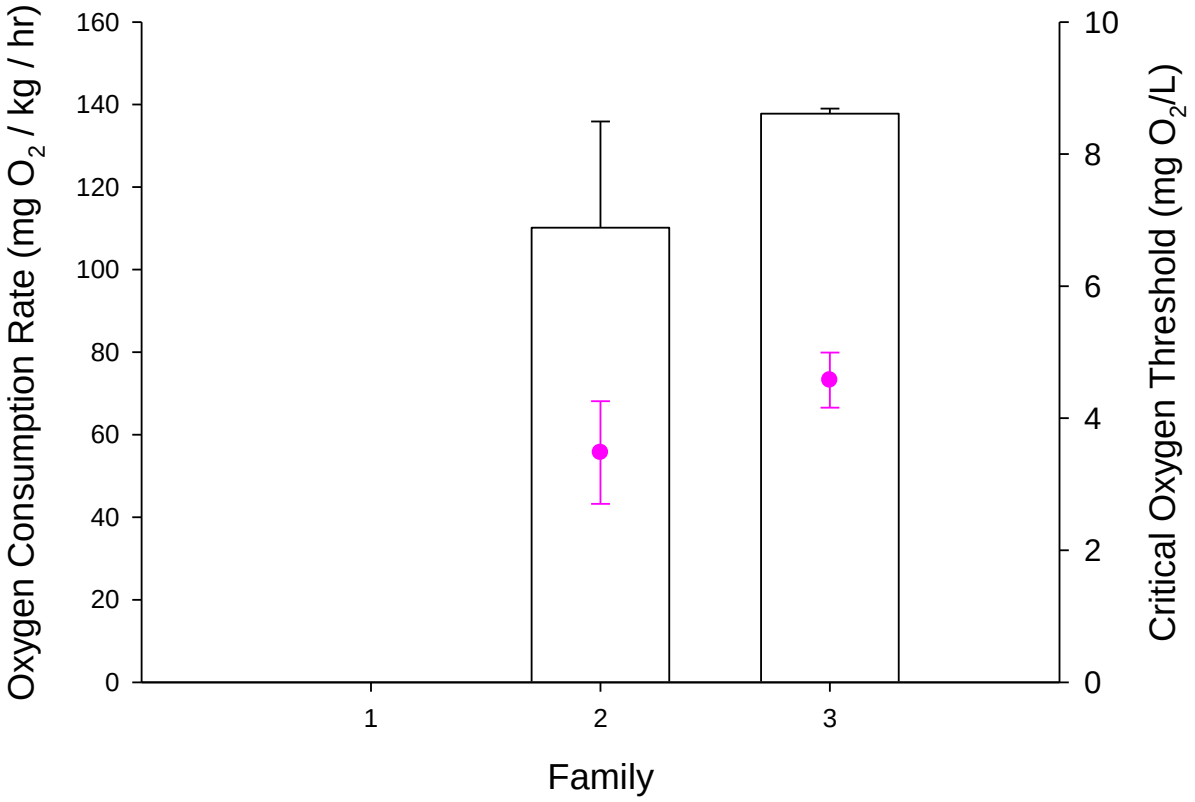

Figure 4. The mean metabolic rates (mg O_2/kg/h, bars) for female regulators from the three families tested in freshwater as well as the mean critical oxygen threshold (mg O_2/L, ●) of females for each family.

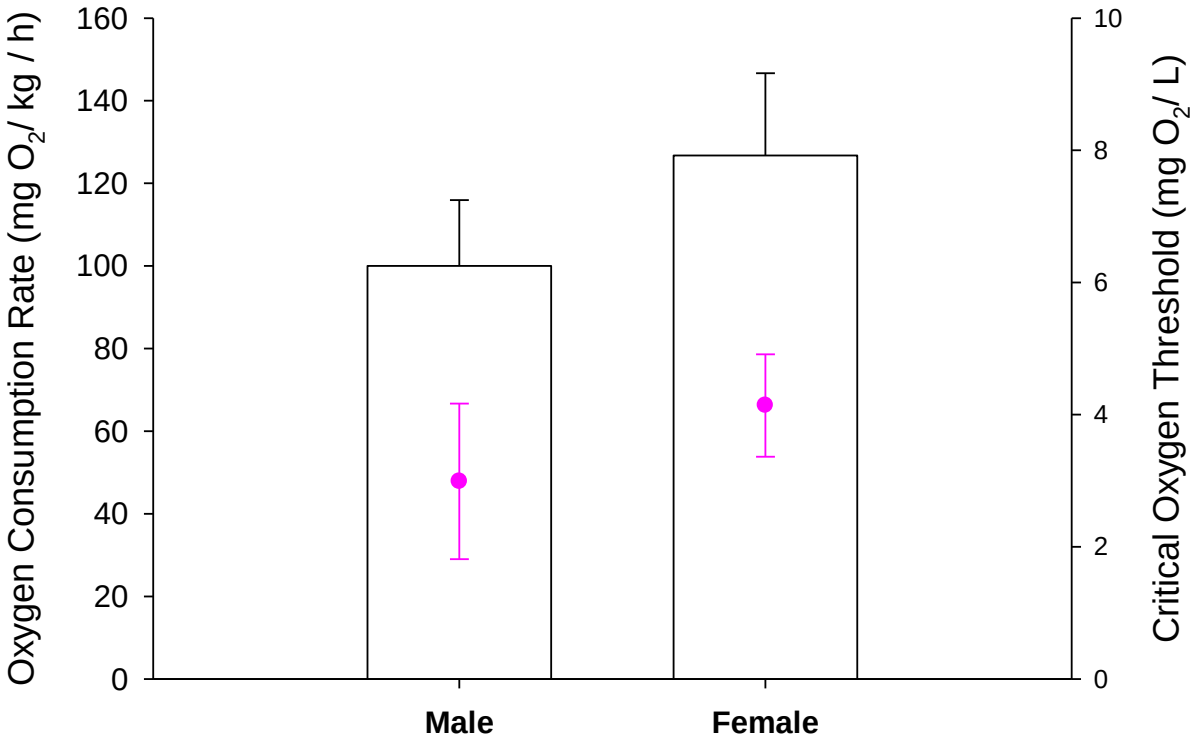

Figure 5. The mean metabolic rate (mg O_2/kg/h, bars) and the mean critical oxygen threshold (mg O_2/L, ●) for male and female regulators combined from the three families tested in freshwater.

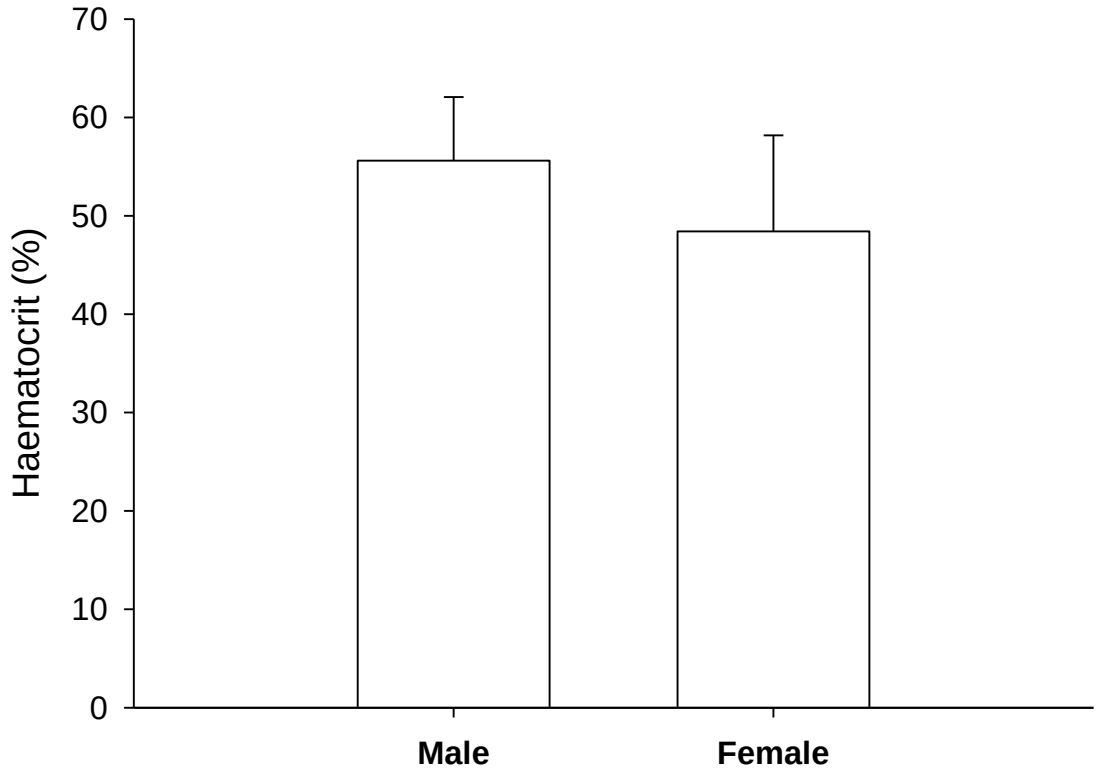

Figure 6. The mean ± standard deviation for haemtocrit (%) for all male and female fish.

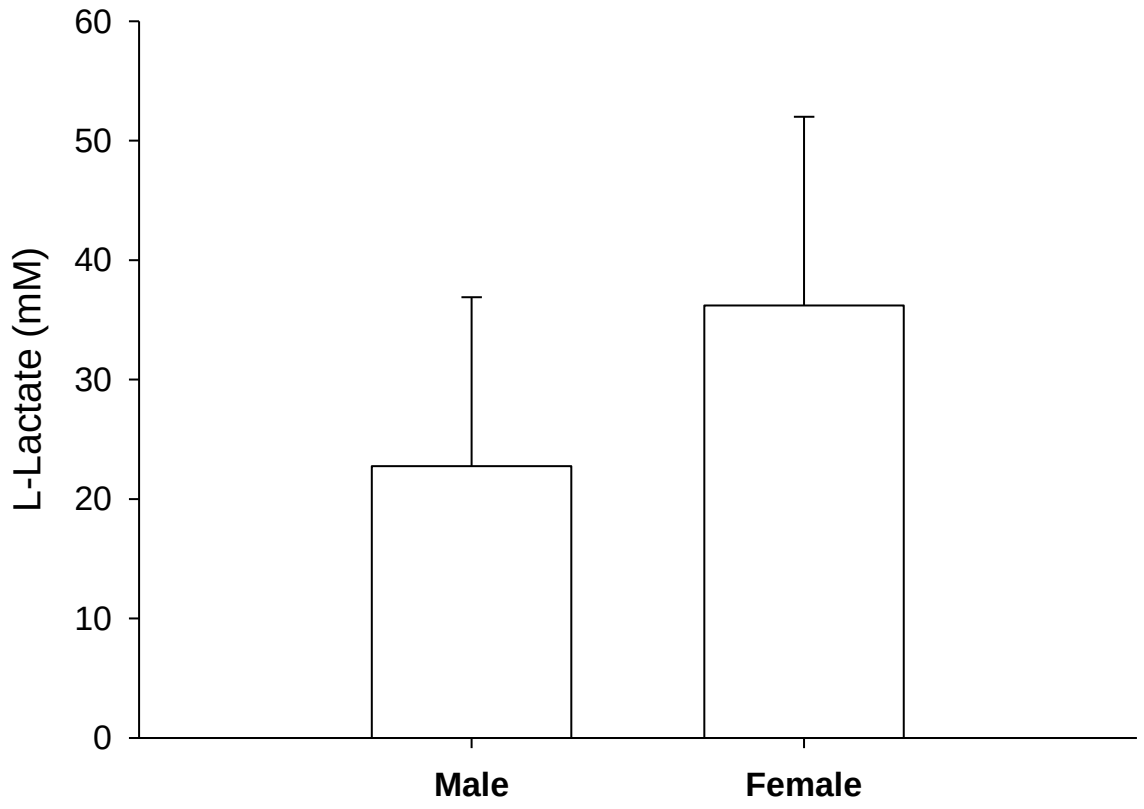

Figure 7. The mean ± standard deviation L-Lactate (mM) for all male and female fish combined from the three families tested.

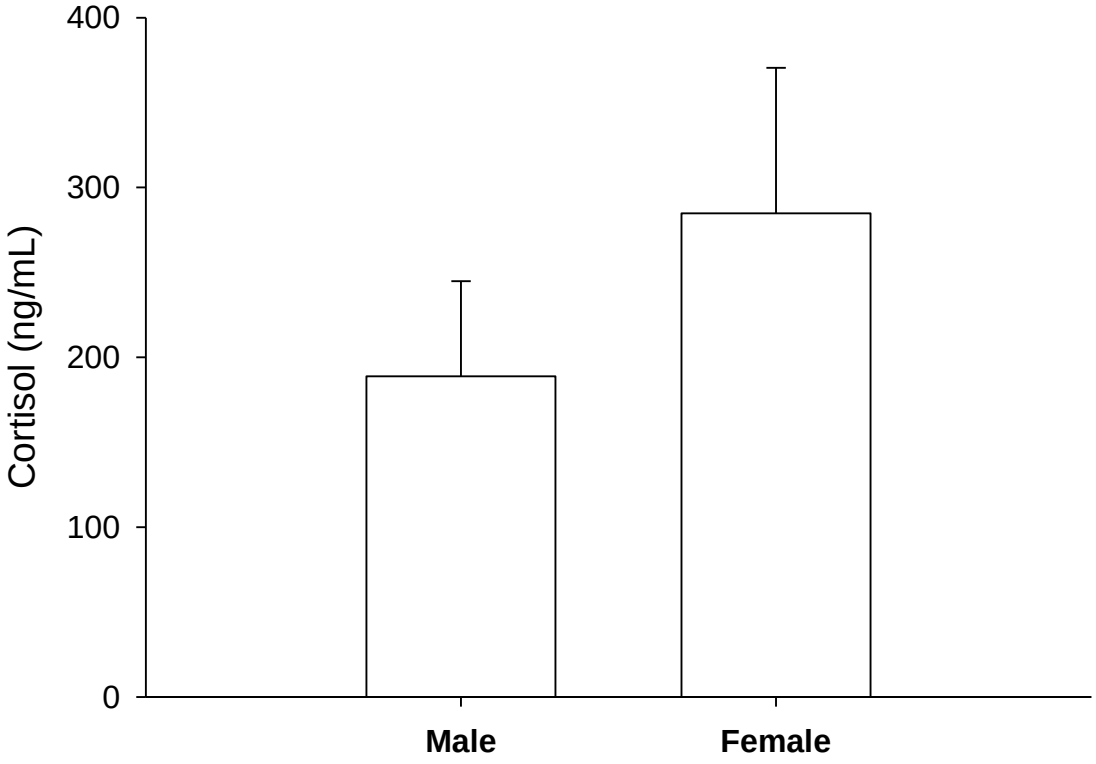

Figure 8. The mean ± standard deviation for plasma cortisol (ng/mL) for all male and female fish combined from the three families tested.

Table 1. Mean values (± standard deviation) for haematology, plasma L-Lactate and cortisol and tissue glycogen for male and female Atlantic salmon, *Salmo salar*, from the three families tested in freshwater (Experiment #2).

Family	**1**	**2**	**3**	
Male				p-value
Haematocrit (%)	48.87[a] ± 1.37	59.81[b] ± 4.90	56.61[a,b] ± 6.33	0.05
Haemaglobin (g/dL)	17.14 ± 1.53	19.40 ± 2.23	18.73 ± 4.73	0.70
MCHC	35.33 ± 2.61	32.32 ± 5.12	33.01 ± 5.31	0.71
L-Lactate (mM)	20.26 ± 10.62	19.14 ± 10.84	22.73 ± 16.16	0.90
Cortisol (ng/mL)	277.49 ± 77.06	169.91 ± 46.97	178.68 ± 42.39	0.28
$Glycogen_{WM}$ (µmol/g)	42.9 ± 0.00	65.04 ± 31.2	27.37 ± 0.07	0.22
$Glycogen_{LIVER}$ (µmol/g)	47.62 [b]± 27.60	7.09 [a]± 3.98	24.70[b] ± 4.89	0.04
$Glycogen_{BRAIN}$ (µmol/g)	71.37 ± 17.11	14.39 ± 1.90	65.90 ± 100.05	0.49
Female				
Haematocrit (%)	54.15 ± 3.67	44.29 ± 6.96	46.67 ± 12.67	0.38
Haemaglobin	20.60 ± 1.91	15.55 ± 0.00	13.70 ± 4.73	0.13
MCHC	36.67 ± 4.69	37.85 ± 0.00	28.98 ± 4.73	0.04
L-Lactate	43.09 ± 7.59	35.65 ± 29.45	31.89 ± 12.52	0.59
Cortisol	308.80 ± 11.47		260.86 ± 64.89	0.56
$Glycogen_{WM}$ (µmol/g)	45.51 ± 41.85	130.86 ± 0.00	28.24 ± 25.55	0.06
$Glycogen_{LIVER}$ (µmol/g)	20.44 ± 28.66	8.71 ± 0.00	26.23 ± 26.87	0.85
$Glycogen_{BRAIN}$ (µmol/g)	12.24 ± 5.71	13.75 ± 0.00	37.15 ± 22.02	0.23

Letters across families indicate a significant difference (P>0.05), no attempt was made to compare males and females or different parameters.

$Glycogen_{WM}$ is the glycogen content in the white muscle

Table 2. Mean values (± standard deviation) for haematology, plasma L-Lactate and cortisol and tissue glycogen compared between males and females (all families combined) for Atlantic salmon, *Salmo salar* in freshwater (Experiment #2)

	Male	***Female***	***t***	***Df***	***p-value***
Haematocrit (%)	55.61 ± 6.46	48.42 ± 9.75	2.27	25	0.032
Haemaglobin (g/dL)	18.63 ± 3.47	15.95 ± 4.88	1.16	23	1.210
MCHC	33.31 ± 4.62	32.77 ± 6.32	0.24	22	0.811
L-Lactate (mM)	22.75 ± 14.14	36.20 ± 15.79	-2.42	27	0.023
Cortisol (ng/mL)	188.77 ± 56.00	284 ± 85.70	-3.05	19	0.007
$Glycogen_{WM}$ (µmol/g)	48.14 ± 27.72	45.38 ± 43.33	0.15	14	0.881
$Glycogen_{LIVER}$ (µmol/g)	24.70 ± 25.66	21.89 ± 24.09	0.24	16	0.814
$Glycogen_{BRAIN}$ (µmol/g)	46.39 ± 64.29	26.58 ± 20.18	0.88	17	0.389

$Glycogen_{WM}$ is the glycogen content in the white muscle.

Seawater (Experiment #6)

Three pedigreed families were examined from 2009 year-class of the SALTAS selective breeding program. Families were selected based on their ranking within the selective breeding program and reflected a range of overall performance levels. These fish had already undergone conditioning for the spawning season and therefore the experiments were conducted on starved fish. All fish that were used in this experiment were ripe males with the exception of one fish which was female and was therefore excluded from the analysis based on experiment #2 where differences between males and females were identified for metabolic rate as well as other key physiological parameters.

Freshwater

Fifty percent of the fish in this experiment were measured in both freshwater and seawater to determine if the pattern of oxygen regulation or oxygen conformation continued through seawater transfer. Of the fish measured in freshwater, 42.9% met all criteria required to be classified as oxygen regulators (Barnes, et al., 2011), 14.3% were oxygen conformers and the remaining 42.9% did not follow a specific

pattern and displayed extremely variable metabolic rates. The mean plateau metabolic rate for the fish which regulated was 98.6 ± 13.2 mg O_2/kg/h. This is nearly identical to the metabolic rate determined for the 2008 year class males. The fish that conformed to the environment had a metabolic rate at 80-100% saturation of 141.4 mg O_2/kg/h. It will be shown throughout this report that oxygen conformers consistently have a higher metabolic rate than oxygen regulators.

Seawater

The three families examined were family 4 (6 fish), 5 (4 fish) and 6 (4 fish). While these are not large numbers of fish, the numbers of fish available from an individual family that have been pedigreed as part of the selective breeding program was limited for this year (B. Evans, personnel communication). Unfortunately, we were not able to test any additional fish (females) from these three families, due to time constraints of the project.

From each family, between 25 and 50% of fish were oxygen regulators. In family #4, 33% of the fish were oxygen regulators and 67% of the fish were oxygen conformers. In family #5, 25% of the fish were oxygen regulators and the remainder of the fish were neither oxygen regulators nor conformers but exhibited widely variable metabolic rates similar to what we observed the freshwater phase of this experiment. Fifty percent of the fish from family #6 were oxygen regulators, one fish was an oxygen conformer and one fish had widely variable metabolic rates. There were apparent differences in the occurrence of regulators among the three families even with the small numbers of fish tested.

The metabolic rates for regulators from each family were not significantly different from each other ($p=0.716$, $df=5$, $F=0.397$) and averaged 136.16 ± 30.05 mg O_2/kg/h (Fig. 9) in seawater. The metabolic rate again was in the range of reported values for adult Atlantic salmon (Enders and Scruton, 2005). The critical oxygen threshold (P_{crit}) was not significantly different among the three families ($p=0.636$, $df=5$, $F=0.572$, Fig. 9) and averaged 3.35 ± 0.85 mg O_2/L. Individual values ranged from 2.70 mg O_2/L to 4.73 mg O_2/L. Haematocrit (HCT) values were significantly different among the families ($p=0.046$, $df=11$, $F=4.419$, Fig. 10). These differences may relate to oxygen carrying capacity and ultimately may indicate differences in robustness

amongst families. Haemaglobin was not significantly different among the three families (p=0.545, df=2, F=0.66; Table 3). No differences were apparent among families for lactate (0.987, df=2, F=0.013) white muscle (p=0.56, df=2. F=0.64) and brain (p=0.86, df=2. F=0.16) glycogen (Table 3). Liver glycogen was again significantly different among the families (p=0.026, df=2, F=8.26), however due to limited samples from family #6 a post-hoc test was not performed (Table 3).

When oxygen regulators and conformers were compared to each other in seawater, significant differences occurred in the white muscle glycogen with oxygen conformers having 2.5 times the glycogen of oxygen regulators (Table 4). This could possibly be a way of distinguishing regulators from conformers. No other significant differences were determined between regulators and conformers (Table 4).

Freshwater to seawater transfer

There was no consistent pattern in the ability of fish to maintain their metabolic category through the seawater transfer. However, 57% of the fish tested in freshwater maintained the pattern exhibited in freshwater through the seawater transfer. Of fish that regulated, only one fish maintained the ability to oxygen regulate in seawater and the metabolic rates were not different between freshwater and seawater (Fig. 11). The one oxygen conformer in the freshwater group, maintained its pattern of conforming in seawater (Fig 12); again, the metabolic rate was not different between freshwater and seawater. It should be noted that the freshwater fish were not allowed to reach the critical oxygen threshold, this was done in order to ensure the fish were not exposed to oxygen concentrations which would have induced stress (based on experiment #3 in freshwater). Of the fish that had widely variable metabolic rates, 66% of these fish continued to have widely variable rates once transferred to seawater.

There were some experimental and time constraints that restricted the numbers of fish available for this experiment. In the previous progressive hypoxia experiment (Experiment #2) we were able to measure the females from the same families giving larger overall family numbers and permitting comparison of male and female fish. This was not possible in the current year because the females for the 2009 year class were not available until June 2011. With the time constraints of this project it

was impossible to complete the freshwater component of the experiment, followed by a 4 week acclimation to seawater and then run the seawater phase by the completion of the project in July 2011. Another constraint with the 2009 year class was that we were limited to 6 males per family (B. Evans, personnel communication). Due to these constraints, our recommendation would be to repeat this experiment next year with the 2010 year class with both males and females and include a wider range of families.

It was an aim of these experiments to determine if oxygen regulation had a genetic component to it. It is clear from the data obtained from Experiments #2 and 6 that differences do occur among the families in both freshwater and seawater for the occurrence of oxygen regulators. We based the categorization to be an oxygen regulator on a series of criteria (Barnes, et al., 2011) making it difficult for a fish to classified as an oxygen regulator. Therefore, individuals which met all criteria of oxygen regulators had to have exhibited this pattern over a number of hours. It was also apparent that fish were able to maintain their metabolic pattern (whether it be as an oxygen regulator, conformer or highly variable) from freshwater through seawater transfer.

It became increasing evident that there is a distinct difference between males and females within a family. It is clear from our results that the individual females we tested show evidence of being less hypoxia tolerant than individual males. Because commercial production can be associated with a high risk of hypoxia (where DO can decrease quite rapidly) due to many factors in both hatcheries and cage culture, including feeding, stocking density, thermoclines and organic loading on water systems (Johansson, et al., 2006; Johansson, et al., 2007; Diaz and Breitburg, 2009), it is the opinion of the authors that this is an area of high importance to be investigated further.

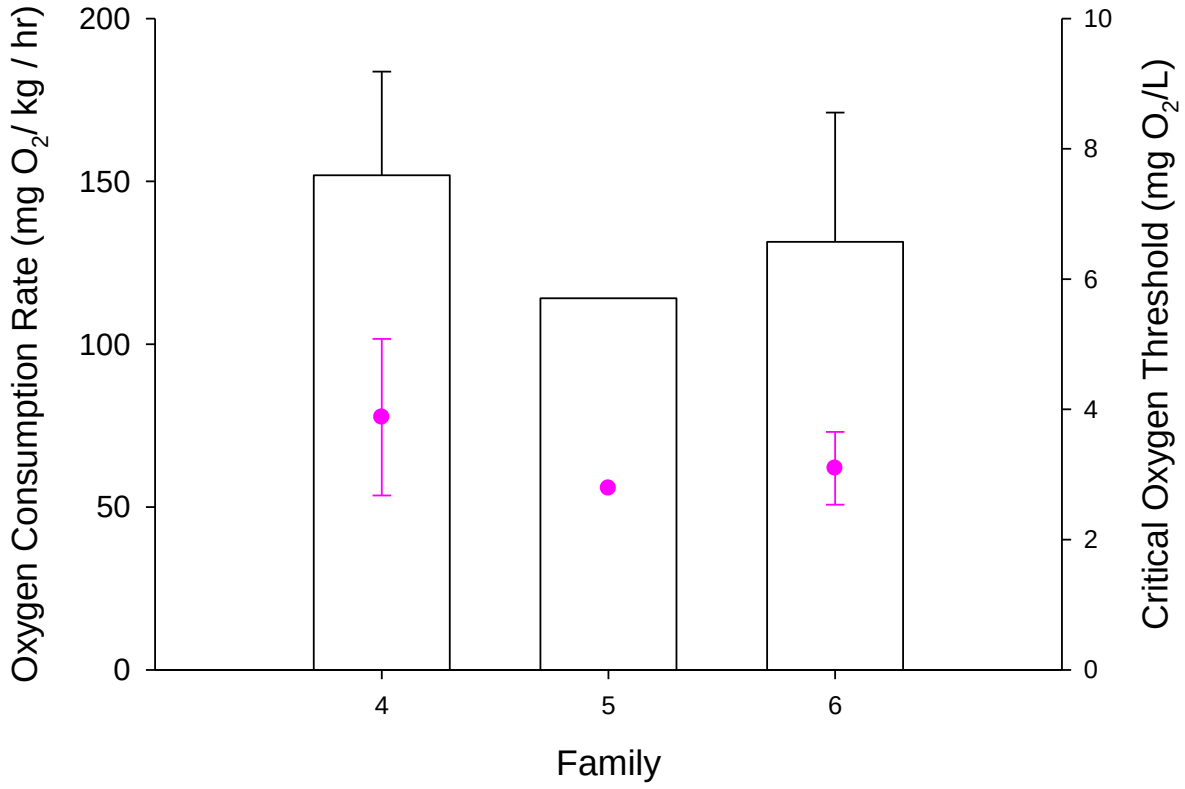

Figure 9. The mean metabolic rates (mg O_2/kg/h, bars) ± standard deviation for regulators from the three families tested in seawater as well as the mean critical oxygen threshold (mg O_2/L, ●) ± standard deviation for each family.

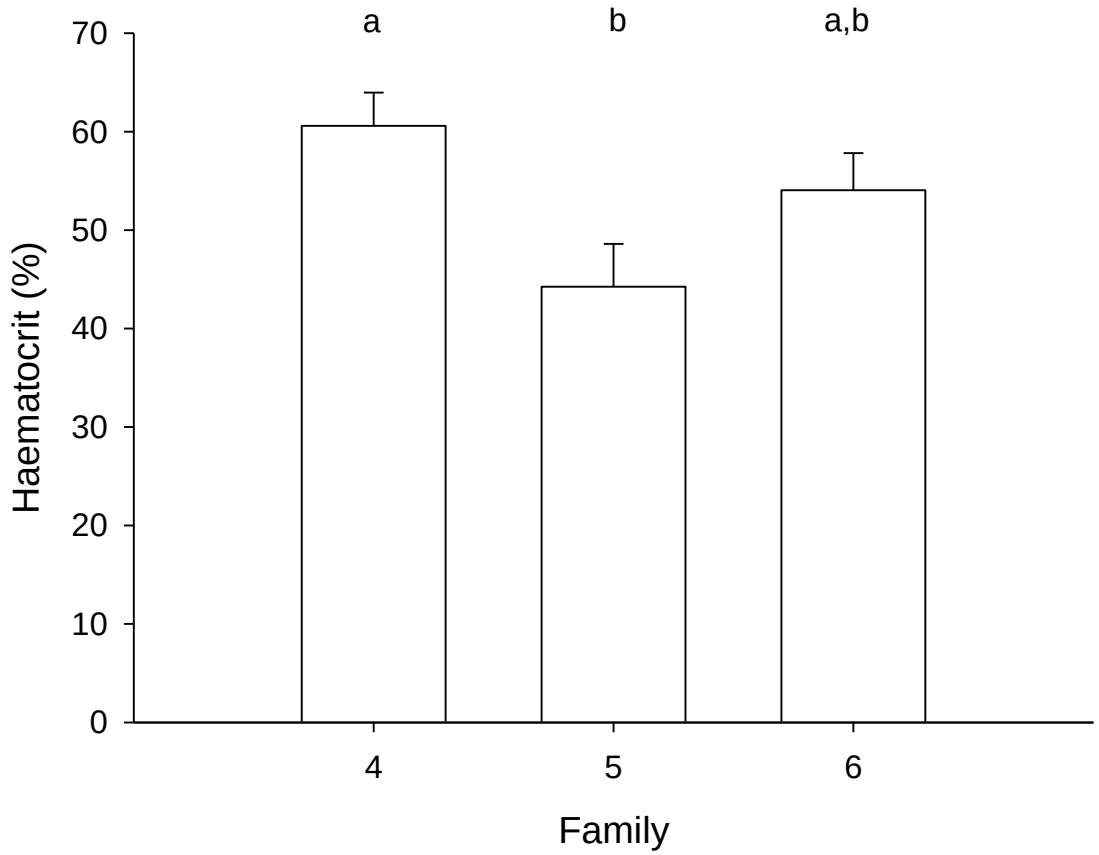

Figure 10. The mean Haematocrit values (%) ± standard error for all fish from the three families tested in seawater.

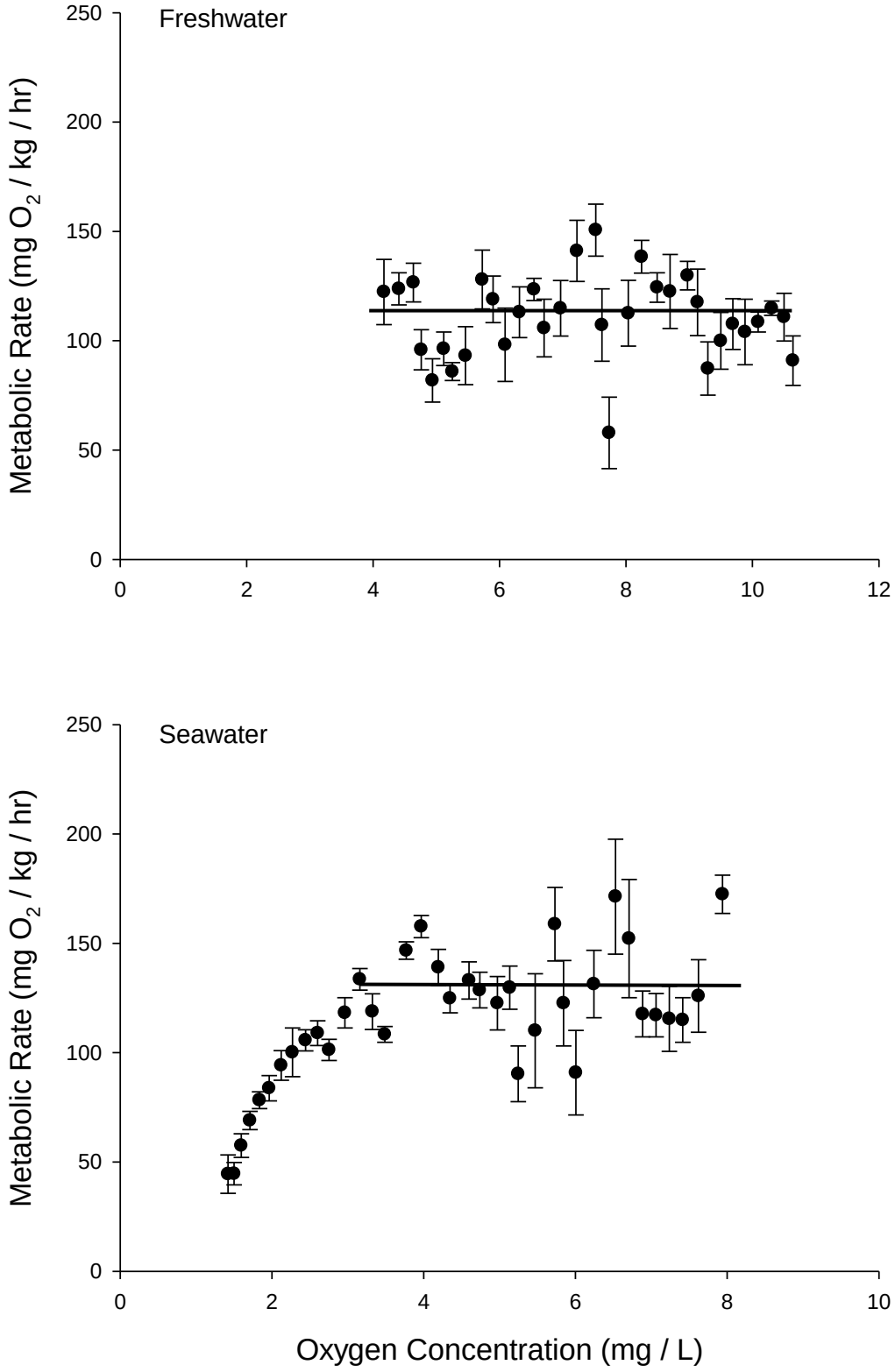

Figure 11. Example of an individual regulator through seawater transfer, each point represents the mean metabolic rate (± standard deviation, mg O_2/kg/hr) over 30 mins. The solid line represents the mean plateau metabolic rate for the regulation period.

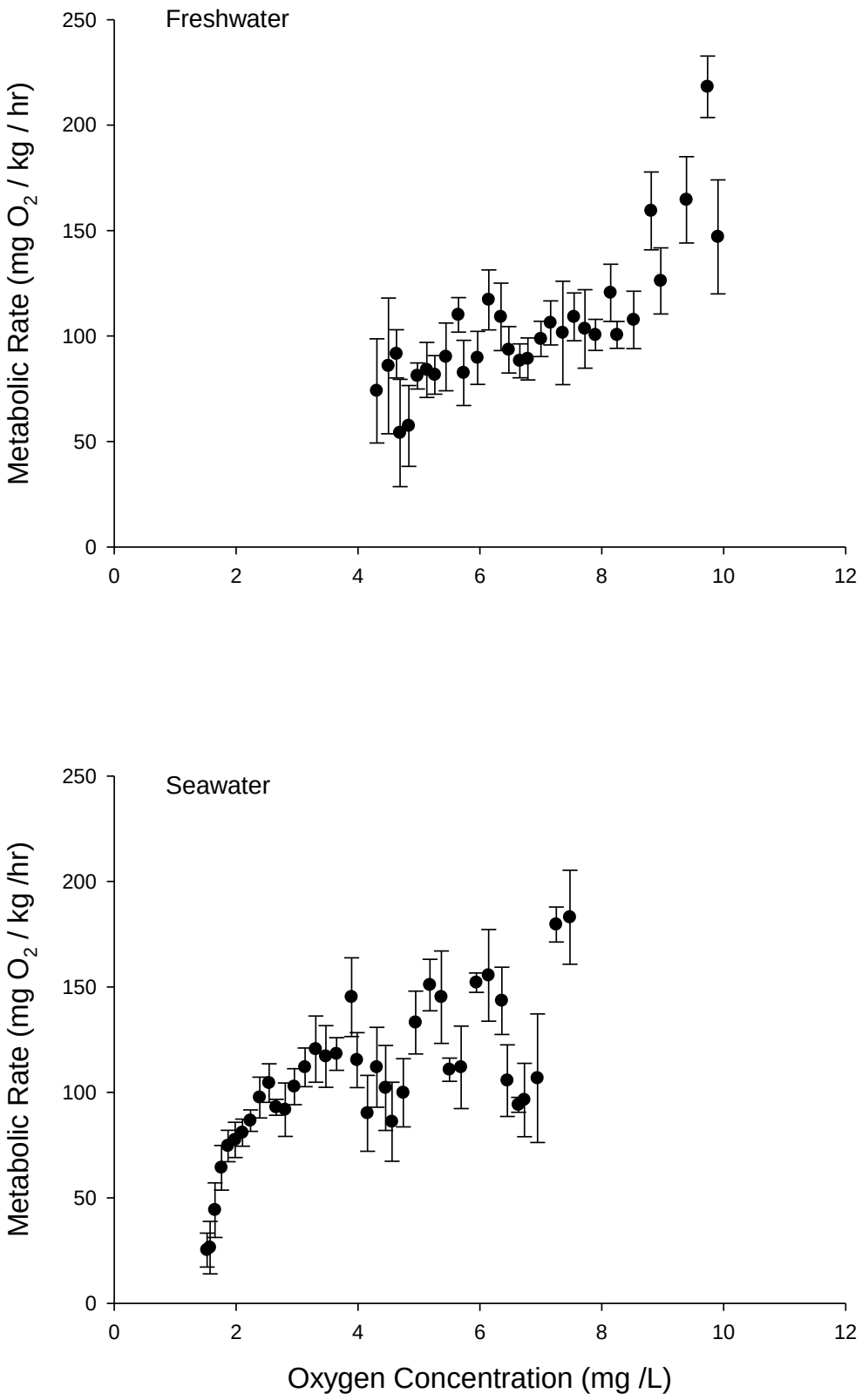

Figure 12. Example of an individual conformer through seawater transfer, each point represents the mean metabolic rate (± standard deviation, mg O_2/kg/hr) over 30 mins.

Table 3. Mean values (± standard deviation) for haematology, plasma L-Lactate and cortisol and tissue glycogen for male Atlantic salmon, *Salmo salar*, from the three families tested in seawater (Experiment #6).

Family	**4**	**5**	**6**	
				p-value
Haematocrit (%)	60.59^{b} ± 5.90	44.24^{a} ± 12.50	$54.05^{a,b}$ ± 4.42	0.046
Haemaglobin (g/dL)	20.90 ± 5.07	17.36 ± 1.69	20.69 ± 2.13	0.345
MCHC	34.35 ± 7.43	37.59 ± 8.45	38.35 ± 3.37	0.461
L-Lactate (mM)	15.41 ± 7.75	14.88 ± 14.55	14.26 ± 12.11	0.987
Cortisol (ng/mL)				
$Glycogen_{WM}$ (µmol/g)	52.45 ± 34.34	32.62 ± 5.13	59.61 ± 39.47	0.561
$Glycogen_{LIVER}$ (µmol/g)	42.98 ± 16.01	16.07 ± 21.31	101.55 ± 0.00	0.026
$Glycogen_{BRAIN}$ (µmol/g)	51.35 ± 22.79	44.68 ± 60.23	61.27 ± 13.84	0.856

Letters accross families indicate a significant difference (P>0.05), no attempt was made to compare parameters within a family.

$Glycogen_{WM}$ is the glycogen content in the white muscle.

Table 4. Mean values (± standard deviation) for metabolic rates, haematology, plasma L-Lactate and cortisol and tissue glycogen for O_2 regulating and O_2 conforming Atlantic salmon, *Salmo salar* in seawater (Experiment #6).

	O_2 Regulators	***O_2 Conformers***	***t***	***df***	***p-value***
Metabolic Rate (mg O_2/kg/hr)	136.16± 30.05	169.12 ± 38.68	-1.05	8	0.171
Haematocrit (%)	51.24 ± 9.09	60.95 ± 6.83	-1.77	7	0.121
Haemaglobin (g/dL)	18.87 ± 4.94	23.14 ± 1.14	-1.68	6	0.143
MCHC	33.38 ± 8.38	37.82 ± 2.60	-1.01	6	0.350
L-Lactate (mM)	16.16 ± 11.89	15.56 ± 7.99	0.83	6	0.936
Cortisol (ng/mL)					
$Glycogen_{WM}$ (µmol/g)	28.84 ± 12.91	72.17 ± 4.87	-4.38	4	0.012
$Glycogen_{LIVER}$ (µmol/g)	32.99 ± 10.81	48.86 ± 13.32	-1.39	3	0.260
$Glycogen_{BRAIN}$ (µmol/g)	42.12 ± 28.58	55.38 ± 23.02	-0.58	5	0.590

$Glycogen_{WM}$ is the glycogen content in the white muscle.

2. Provide an industry definition of a hypoxic event and link to management practice

These experiments attempted to provide industry with a definition of a hypoxic event in both freshwater and seawater by examining a wide range of parameters along an oxygen gradient (from ~100% saturation to the point where fish lost equilibrium). The experiment was designed to sample the fish continuously along the oxygen gradient, in order to identify whether there was a “break point” in any of the physiological parameters. This “break point” would then provide a parameter that could be measured to correlate to the industry definition of a hypoxic event. To our knowledge the measurement of physiological changes in relation to an oxygen gradient has not been presented in the literature previously. The majority of literature focuses on exposing animals to extreme hypoxic conditions and measuring their response (Dunn and Hochachka, 1986; Richards, et al., 2007; Farrell and Richards, 2009). Our aim was to gain an understanding of the physiological changes which occur along the oxygen gradient. This approach was potentially more useful to industry because it would have identified a useful parameter to measure and correlate with physiological status of fish (preferably before a hypoxic event).

This was the first time an experiment of this scale was conducted in both freshwater and seawater on Atlantic salmon and therefore a wide range of parameters was measured in order to develop a database of information which we could build upon in the future. Due do the large numbers of fish needed for this experiment, fish from the general population at SALTAS were used. We initially proposed to repeat this experiment under high (summer) temperature, however the original grant was pared back by industry and this experiment was only run at 13.5°C. Nevertheless it provides excellent baseline data to later build upon.

In freshwater, SALTAS has currently set oxygen alarms on all systems to alert when the dissolved oxygen reaches 6 mg O_2/L in order to prevent the fish from being exposed to hypoxic conditions. In seawater, it is routine for farms to stop feeding when the dissolved oxygen reaches a certain level. At Huon Aquaculture this level is 5.0 mg O_2/L (D. Mitchell, personnel communication) while at Tassal they will feed

under restrictions until 6.0 mg O_2/L (L. Hubbert, personnel communication). These levels depend on a number of factors such as stocking densities, tide and current conditions. In both the freshwater and seawater phases of Atlantic salmon culture in Tasmania, ideal oxygen concentrations should remain above 7 mg O_2/L to avoid any negative growth or feed conversion issues (D. Mitchell and L. Hubbart, personnel communications). It is therefore essential to understand when certain physiological and biochemical changes are occurring along the oxygen gradient in both freshwater and seawater and to determine if they occur above or below the thresholds already set by industry.

Freshwater (Experiment #3)

Fish from SALTAS' general population were examined in this experiment. Fish were maintained in freshwater for the duration of this experiment. An initial 10 fish were sampled at initial dissolved oxygen (DO) concentration of 8.9 mg O_2/L (13.5°C) before the water flow and the aeration to the tank were turned off. Fish were sampled along the oxygen gradient until fish started to lose their equilibrium at 1.8 mg O_2/L, at this point the final six fish were sampled. A control tank which was maintained at a saturated level of oxygen was sampled four times over the course of the experiment.

Blood gas

Understanding the changes which occur in the blood gases along the DO gradient was an area of interest for the authors and was explored extensively. Blood oxygen concentration was measured using a technique described by Cech Jr. (1990). This is not the standard technique used in the literature for measuring blood gases. Blood gas is generally measured by cannulising the fish and holding the fish individually and/ or restraining the fish to a relatively small area (generally so they don't knock the catheter out). Samples are then taken in a closed loop to read the blood gas (Stevens and Randall, 1967; Gamperl, et al., 1994; Leef, et al., 2007; Kristensen, et al., 2010). This type of measurement would not have been appropriate for these experiments (where we destructively sampled a large number of fish over the DO gradient) or, more importantly, for any future on-farm

measurements. Therefore, we decided to explore an alternative method of direct blood sampling from the dorsal aorta. Results were varied among the experiments, however, after a successful calibration in freshwater the DO content in the blood was extremely low and the results showed no significant change along the oxygen gradient ($F=0.27$, $df= 65$, $p=0.61$). On average the blood oxygen content was 18.80 ± 8.99 mmHg, this is approximately 12% saturated and did not change along the oxygen gradient. Based upon the successful calibration, these results were unexpected. It is our view that further investigation into alternative methods for blood sampling would be beneficial to the industry as this is an important aspect in defining the onset of hypoxia. Such a study was beyond the scope of the present limited grant.

Blood pH did not change along the DO gradient ($F=1.29$, $df= 65$, $p=0.26$) and averaged 7.39 ± 0.19. These results are within the range of reported values for blood pH in the literature for freshwater salmonids (Holeton and Randall, 1967; Hosfeld, et al., 2010). Blood gas and pH were not measured on the control fish.

Haematology

The results showed that significant changes occurred over the DO gradient for haematocrit and this was expressed by the equation $HCT = -2.21DO + 59.22$ ($F=38.54$, $df=67$, $p<0.001$, Fig. 13). Further analysis of the data using a break point analysis to determine the point where the physiological changes occur along the oxygen gradient indicate that the first signs of a hypoxic event appear at 5.37 mg/L in freshwater where a change in haematocrit occurs (Fig 13). At dissolved oxygen levels above the break point, HCT increased as DO decreased. After the breakpoint the HCT levelled off and indicated that during mild hypoxia fish increased the proportion of RBC in their blood, presumably to cope with the decreasing oxygen levels. It has been shown in the literature that fish which had been exposed to hypoxic conditions have a significantly higher HCT level when compared to fish at normoxic levels (Wells, et al., 1989). The control fish had an average HCT value of 41.54 ± 8.81% and is similar to fish prior to the point where the physiological changes occur.

Whole blood haemoglobin (17.91± 5.13 g/dL) did not change (F=.010, df=67, p=0.92) over the DO gradient indicating that the blood's carrying capacity for oxygen remains constant as the fish undergo hypoxic conditions. The control fish had similar haemoglobin values which averaged 18.10 ± 4.68 g/dL. However, when the HCT and haemaglobin are combined to express the mean corpuscular haemoglobin concentration (MCHC) there is a significant decrease in the MCHC as the oxygen decreases in the environment (Fig. 14). The MCHC is the mean concentration of haemoglobin in the red blood cells as determined by equation 2 (above). This is a measurement which is often used in the literature (Sadler, et al., 2000a; Leef, et al., 2007) and therefore we evaluated its use in these experiments. Haemoglobin did not change over the DO gradient so that a clear "break point" in the HCT was obscured and there was high variability amongst fish. Consequently it was not possible to identify a clear break point in the MCHC data either (Fig. 14).

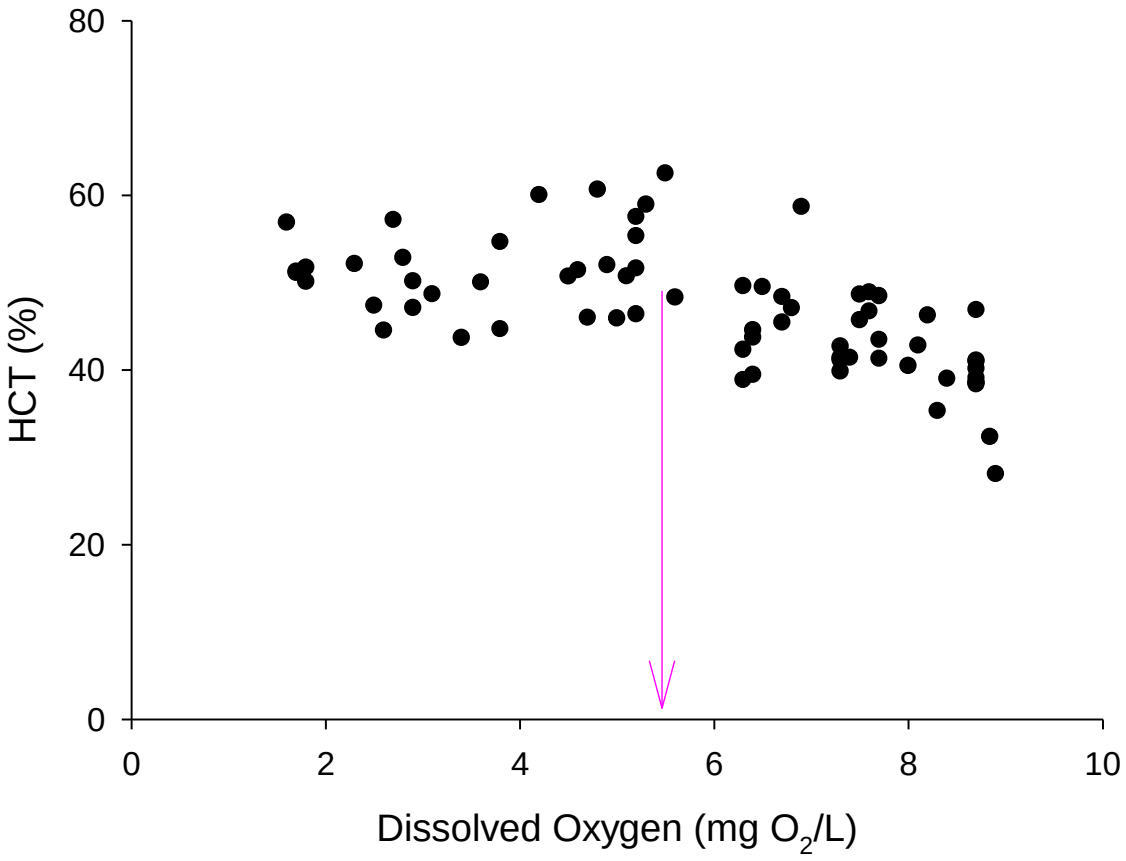

Figure 13. The relationship between the Haematocrit (HCT, %) and the oxygen concentration (mg O_2/L)in freshwater for Atlantic salmon can be determined by the equation HCT (%) = -1.879*DO+57.801 (F=41.42, df=68, p<0.001, r^2=0.379). The arrow indicates the DO concentration (5.37 mg O_2/L) where the physiological change occured and HCT reached a plateau.

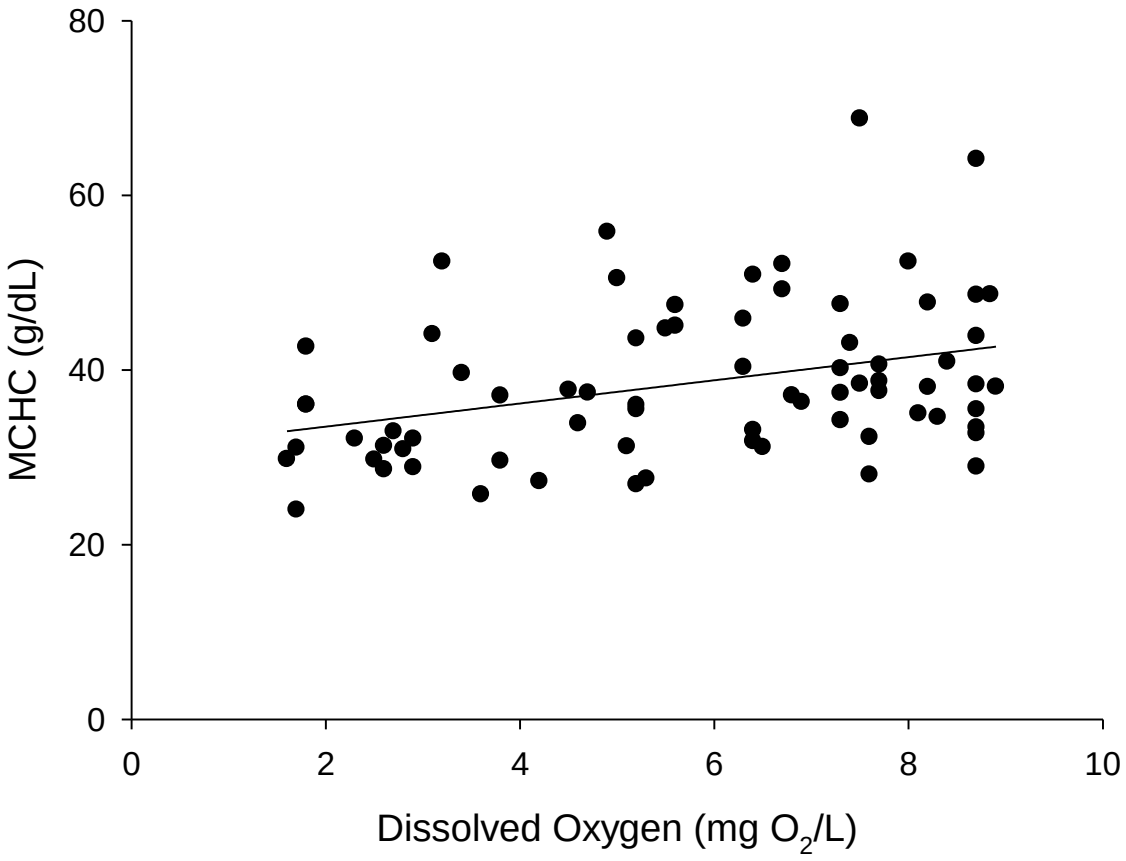

Figure 14. The relationship between the mean corpuscular haemoglobin concentration (MCHC, g/dL) and the oxygen concentration (mg O_2/L) for Atlantic salmon in freshwater can be determined by the equation MCHC = 1.32*DO+30.87 ($F=11.25$, $df=67$, $p<0.001$).

Cortisol, Lactate and Glycogen

The cortisol results show that fish do not exhibit a strong stress response until the oxygen saturation falls below 40% saturation. The cortisol levels of the control fish (28.36 ± 7.62 ng/mL) were constant over the sampling period while at the termination of the experiment the cortisol levels in the hypoxic fish had increased significantly by more than an order of magnitude (264.27 ± 69.58 ng/mL; Fig. 15). Further analysis of the raw data using a break point analysis to determine the point where the physiological changes occur along the oxygen gradient indicated that the cortisol levels did not significantly change until the fish reached an DO level of 3.61 mg O_2/L (at 13.5°C, this is at 34.6% saturation) in freshwater, Fig. 16). Fish in the control tank were within the limits and would be considered unstressed, however some

individual fish in the experimental tank had cortisol above 40 ng/mL and would be considered moderately stressed (Trenzado, et al., 2008). The occurrence of individuals with moderately high cortisol and stress is not surprising given the impact on individual stress responses of other factors such as social hierarchies and previous history. The main finding is that high cortisol and therefore highly stressed fish (R. Wilkinson, personnel communication) are not found until below the break point of 3.61 mg O_2/L.

Plasma lactate results showed a significant relationship over the oxygen gradient; however due to high variability no clear biological relationship can be determined and lactate averaged 4.91 ± 6.85 mM. Overall, this was higher than the control group which averaged 2.41 ± 1.84 mM over the span of the experiment.

No significant relationships were determined for glycogen in the liver ($F=1.37$, $df=51$, $p=0.248$) or brain ($F=1.32$, $df=53$, $p=0.256$) and averaged 123.30 ± 192.55 and 40.33 ± 35.99 µmol/g, respectively. Glycogen in the white muscle significantly decreased with decreasing oxygen concentration and can be expressed by the equation $Glycogen_{WM}$ (µmol/g) = 4.00DO + 19.63 ($r^2 = 0.19$; $F=12.17$, $df=53$, $p=0.001$; Fig. 17). Dunn and Hochachka (1986) have also found that glycogen concentrations in the white muscle decrease during hypoxia exposure. This is explained by the use of glycogen to fuel anaerobic metabolism of glucose via glycolysis. Due to the individual variability of fish, it was not possible to identify a clear break point in the data which would define a point at which a change in white muscle glycogen occurred. Glycogen metabolism may therefore progress throughout the oxygen gradient rather than be triggered at a specific DO. However, the analysis is complicated by not being able to measure the initial glycogen content of each fish, the initial content would have differed between individuals and provided lesser or greater amounts for anaerobic metabolism. The ability of different animals to accumulate glycogen in tissues is viewed as an adaptation to survive hypoxia, it is therefore interesting to note that regulators tend to have lower muscle glycogen than conformers (Tables 3 and 6). It is tempting to conclude this is because glycogen was used by the regulators as DO decreased where as it was not used to the same extent by conformers. It is also interesting to note that different families appear to have different glycogen levels (Tables 1 and 3). However, the complexities around

glycogen accumulation and the importance of individual differences in nutritional status that govern “fuel” stores should not be overlooked. This is clearly an aspect of salmon physiology that would benefit from further investigation.

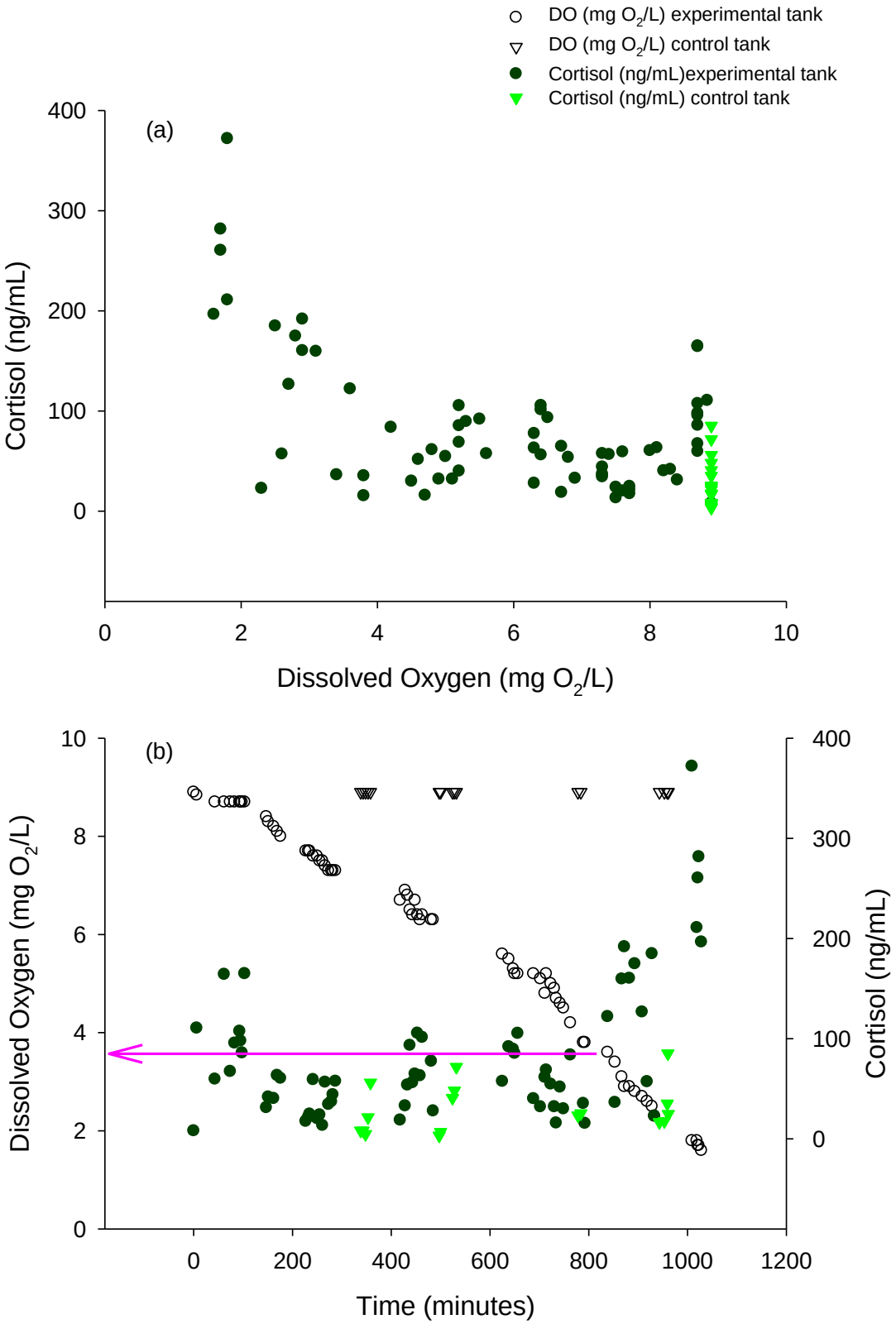

Figure 16. The relationship between plasma cortisol (ng/mL) and the dissolved oxygen (mg/L) for the experimental and control tanks (a). Dissolved oxygen (mg/L) and plasma cortisol (ng/mL) for the experimental and control tank over the time course of the experiment (b). The arrow indicates the critical oxygen level where cortisol levels for Atlantic salmon, significantly changed at 3.61 mg/L in freshwater.

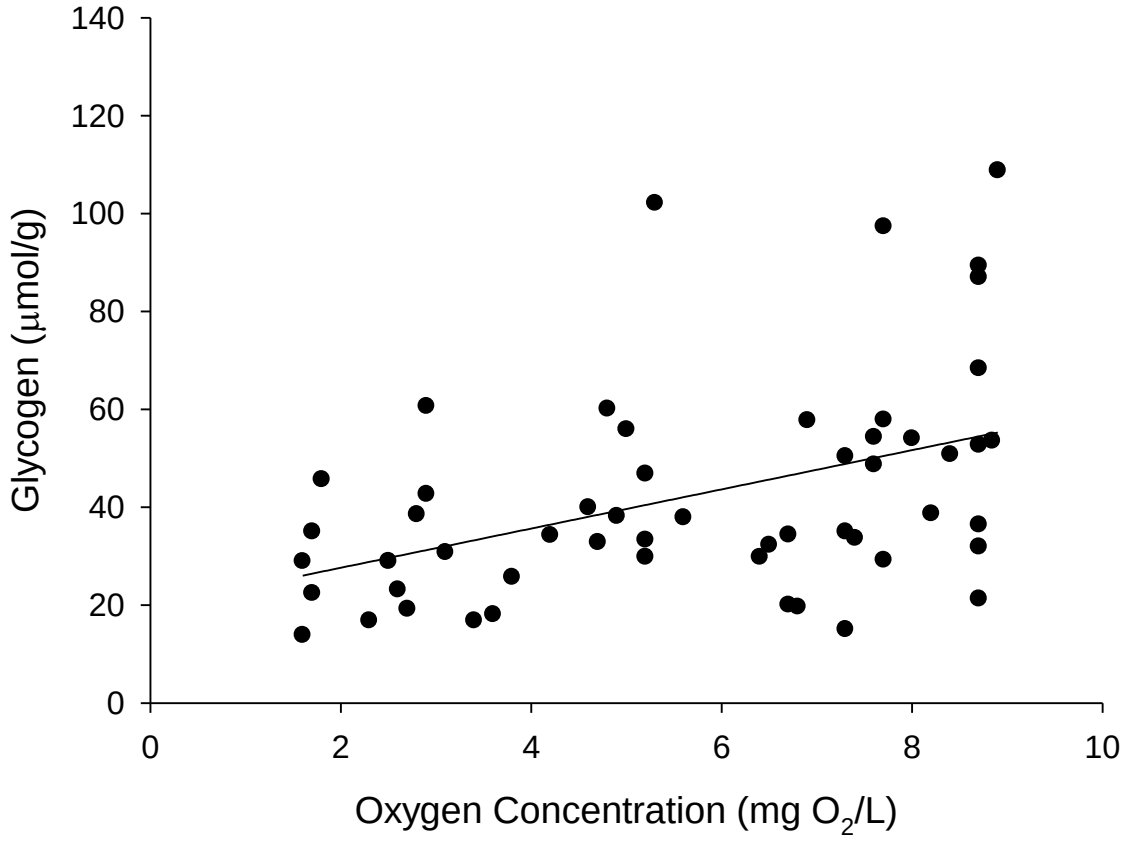

Figure 17. The relationship between white muscle glycogen (µmol/g) and the dissolved oxygen concentration (mg O_2 /L) for Atlantic salmon in freshwater can be determined by the equation $Glycogen_{WM}$ = 4.00*DO+19.632 (F=12.167, df=52, $p<0.001$, $r^2 = 0.190$).

Seawater (Experiment #6)

This experiment is a repeat of experiment #3 except it was conducted in seawater. Fish were held in full strength seawater to determine if a hypoxic event was different for fish which had recently been transferred to sea. This experiment was run at a slightly cooler temperature of 11.6 ± 0.42°C. An initial group of fish were sampled before the aeration and flow to the test tank was shut off and thereafter at intervals along the oxygen gradient which decreased from 10.83 mg O_2/L to 2.37 mg O_2/L. At 2.37 mg O_2/L, the first fish in the tank started to lose their equilibrium and were sampled at this time. Groups of five fish were sampled from the control tank for

haematology, cortisol and tissue metabolites four times over the course of the experiment. To serve as a proper control tank, the control fish were disturbed an equal number of times to test fish in the test tank.

Blood gas

While the blood gas results did not work in the freshwater experiment (above), we achieved better results when this experiment was repeated using the identical technique. The blood oxygen content was still consistently low (on average ~ 30% saturation) compared to values expected from the literature. However, it was possible to determine a significant relationship between the arterial blood oxygen content (mm Hg) and the DO of the experimental tank which is presented below (Fig. 18). It should be noted again, that the method employed in these studies used a non-standard technique and the results should be treated with caution. Values of blood oxygen content in the literature using cannulised fish are often at levels approaching 70% saturation (Powell, et al., 2000; Leef, et al., 2007; Kristensen, et al., 2010). Again, it is our view that further investigation into alternative methods as well as comparisons between new and established methods for blood sampling would be beneficial to the industry because this is an important aspect in defining the onset of hypoxia

Blood gas results showed a linear decrease in blood oxygen content as the oxygen content of the water decreased (Fig. 18) and can be expressed by the equation, Blood oxygen content (mmHg) = 2.54DO + 28.787 ($F=37.91$, $df=84$, $p<0.001$, $r^2=0.311$). This relationship is similar to that determined before for cannulised Atlantic salmon (Powell, et al., 2000). There was no relationship between water DO and blood pH ($F=0.004$, $df=85$, $p = 0.940$) therefore the data were combined and the average blood pH was determined to be 7.64±0.35 over the course of the experiment which is in the range of published literature for Atlantic salmon in seawater (Powell, et al., 2000; Hosfeld, et al., 2009). Blood pH measurements were also made on another pH meter (ISFET, Mini Lab Model IQ125) to confirm our results using the Powerlab®. When the blood pH results were compared to the FW data set (experiment #2 defining hypoxia in FW, mean pH: 7.38±0.19) a significantly higher pH was determined for the seawater acclimated fish ($F=22.71$, $df=177$, $p<0.001$), neither experiment showed any change in pH over the oxygen gradient.

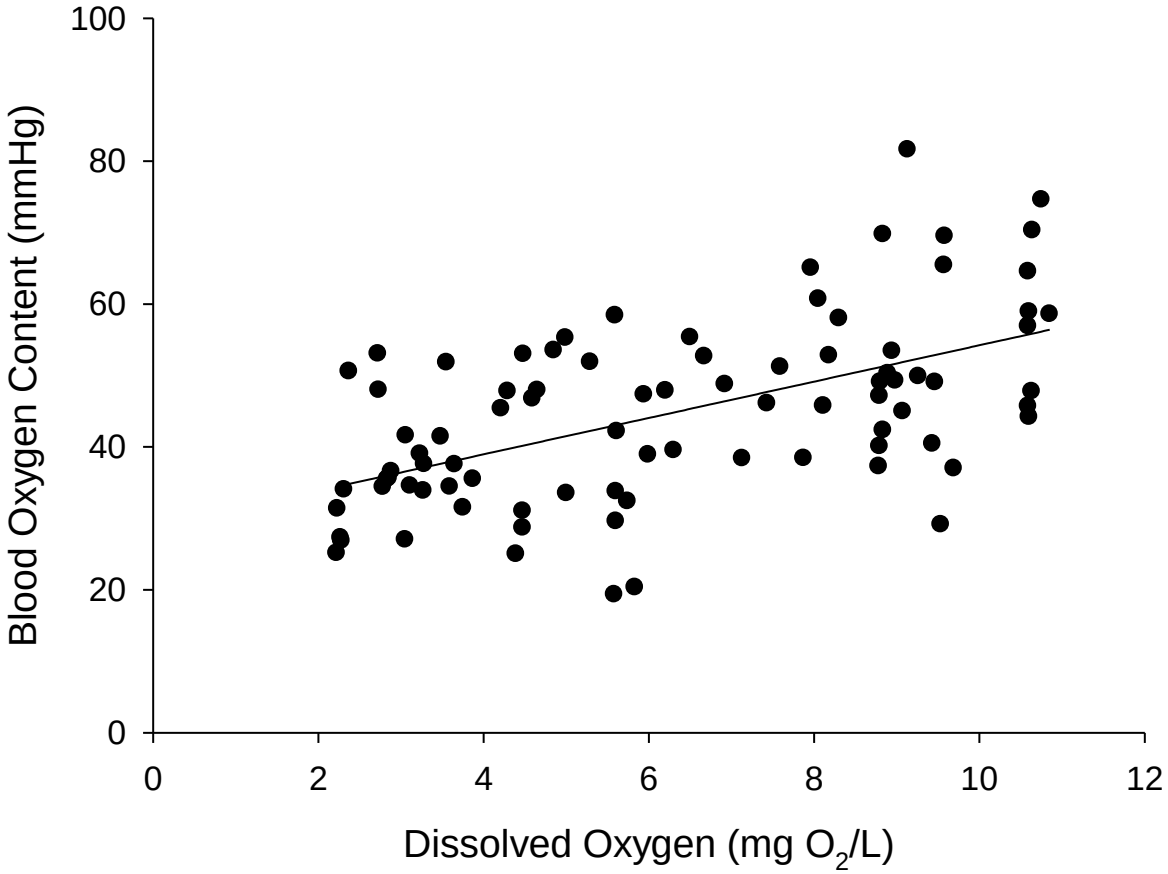

Figure 18. The relationship between the mean blood oxygen content (mmHg) and the dissolved oxygen concentration (mg O_2 / L) in seawater for Atlantic salmon can be determined by the equation Blood oxygen content (mmHg) = 2.54*DO+28.787 ($F=37.91$, $df=84$, $p<0.001$, $r^2=0.311$).

Haematology

Whole blood haemoglobin (15.81 ± 3.10 g/dL) did not change ($F=0.082$, $df=86$, $p=0.775$) over the dissolved oxygen gradient indicating that the blood's carrying capacity for oxygen remains constant as the fish undergo increasingly hypoxic conditions. As the DO concentration decreased in the water, the haematocrit (HCT) of the blood increased and was explained by the equation HCT (%) = -1.263*DO+53.253 (Fig. 19). A break point analysis revealed that at an oxygen concentration of 3.69 mg O_2/L is where the physiological change occurred for HCT and reached a plateau (Fig. 19). This is similar to the observations made in Experiment 3. However, the break point was found at a much higher dissolved oxygen concentration in freshwater (5.37 mg O_2/L). When HCT and haemaglobin are combined to express the mean corpuscular haemoglobin concentration (MCHC)

there is a significant decrease in the MCHC as the oxygen decreases in the environment which can be explained by the equation MCHC (g/dL) = 1.104*DO+28.656 (F= 12.753, df=86, p=0.00, r^2=0.129; Fig. 20).

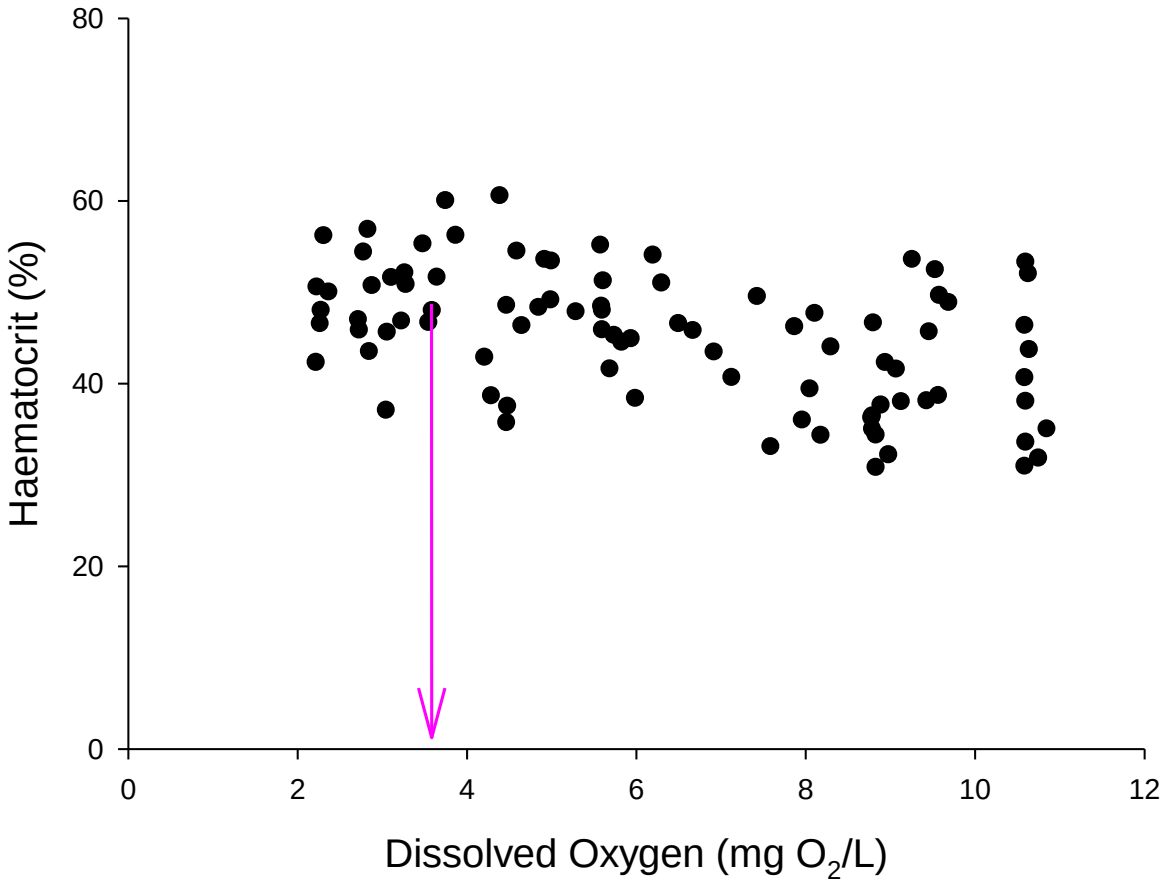

Figure 19. The relationship between the Haematocrit (HCT, %) and the oxygen concentration (mg O_2/L) in seawater for Atlantic salmon can be determined by the equation HCT (%) = -1.263*DO+53.253 (F=26.746, df=87, p<0.001, r^2=0.235). The arrow indicates the DO concentration (3.69 mg O_2/L) where the physiological change occurred and HCT reached a plateau.

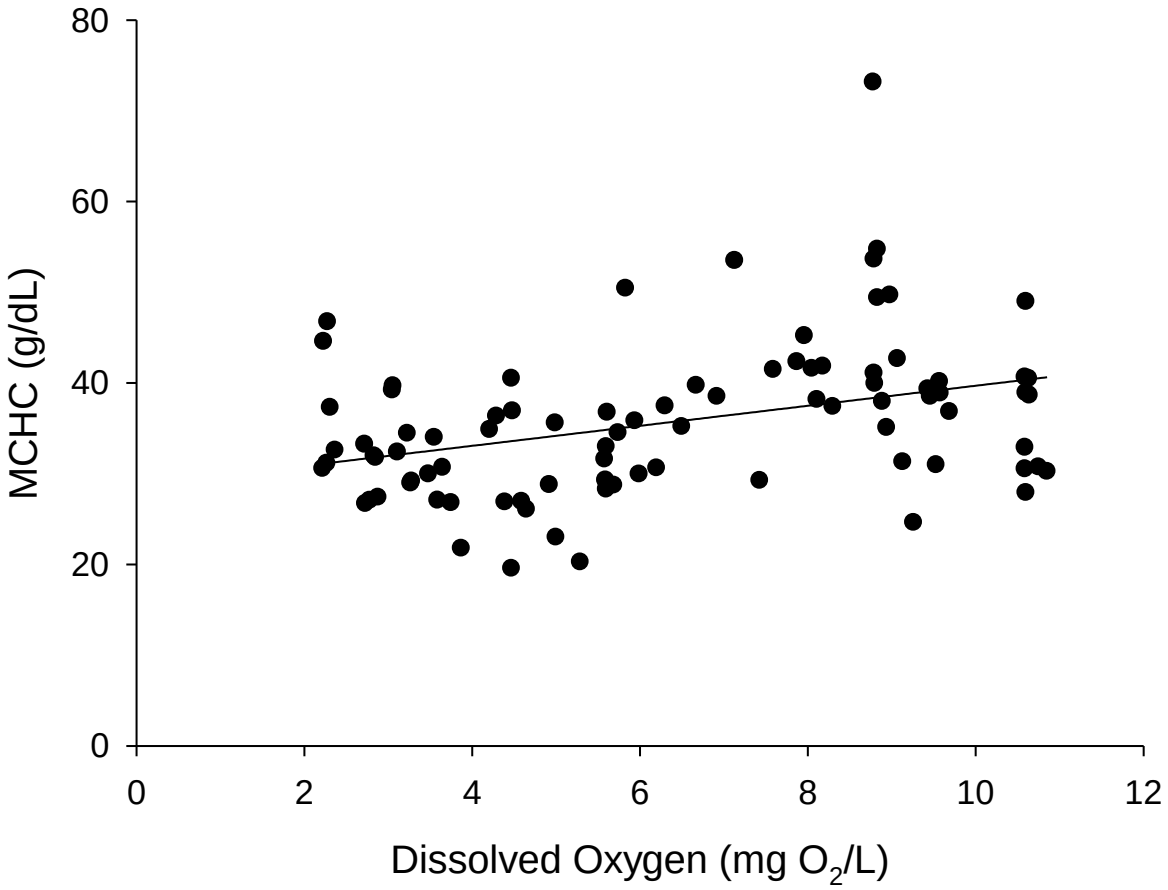

Figure 20. The relationship between the mean corpuscular haemoglobin concentration (MCHC) and the oxygen concentration (mg O_2/L) in seawater for Atlantic salmon can be determined by the equation MCHC (g/dL) = 1.104*DO+28.656 (F= 12.753, df=86, p=0.00, r^2=0.129).

Cortisol, Lactate and Glycogen

The cortisol results show that the fish do not exhibit a stress response until the oxygen saturation falls below 50% saturation. The breakpoint analysis identified a DO of 4.27 mg O_2/L as the point where the cortisol level increased significantly. At 11.6 °C this is 47% saturation. The cortisol levels of the control tank were constant over the sampling period and averaged 20.43 ± 14.68 ng/mL while at the termination of the experiment the cortisol levels of the hypoxia fish increased significantly to a level nearly an order of magnitude higher (184.82 ± 32.17 mg/mL; Fig. 21). The cortisol levels in the control tank are at levels which reflect unstressed fish (Trenzado, et al., 2008). When these results are compared to Experiment #2 above,

it is clear that fish which have been recently transferred to sea are more susceptible to high stress levels associated with hypoxia.

Plasma lactate results did not show a significant relationship over the dissolved oxygen gradient and averaged 5.36 ± 7.81 mM. Overall, this was higher than the control group which averaged 1.16 ± 1.21 mM over the span of the experiment.

There were no relationships between DO and either white muscle glycogen ($F=1.583$, $df=53$, $p=0.214$) or liver glycogen ($F=1.287$, $df=51$, $p=0.262$) which averaged 77.04 ± 50.25 and 117.53 ± 112.32 µmol/g, respectively. Whilst there was a significant relationship between brain glycogen as expressed by $Glycogen_{Brain} = -14.068DO + 188.27$ ($F=8.815$, $df=57$, $p=0.004$, $r^2=0.134$; Fig. 22). Although there is limited evidence from rats that pre-conditioning to hypoxia increases brain glycogen (Brucklacher, et al., 2002), the present relationship may reflect the presence of 3 outliers.

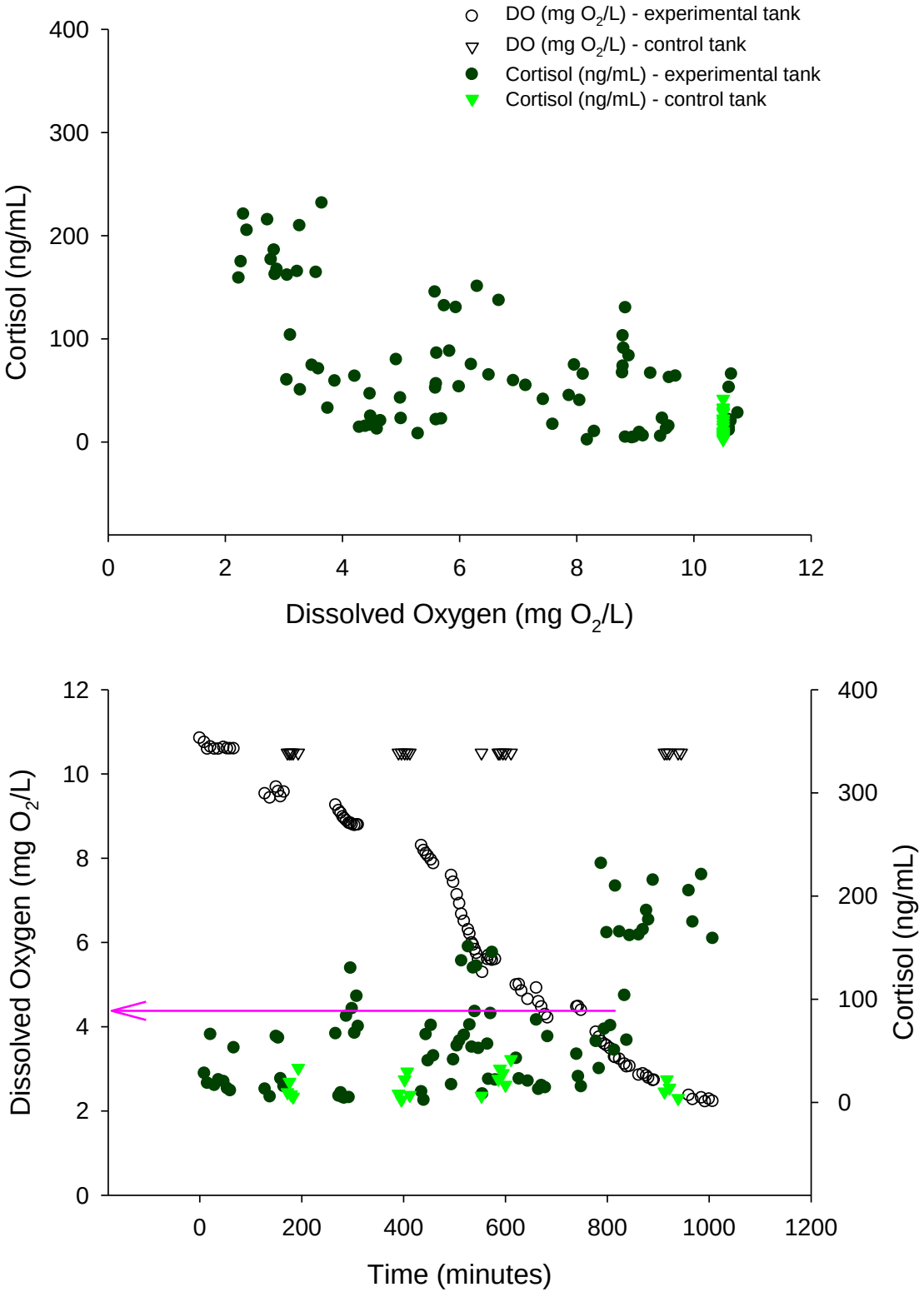

Figure 21. The relationship between plasma cortisol (ng/mL) and the dissolved oxygen (mg O_2/L) for the experimental and control tanks (a). Dissolved oxygen (mg O_2/L) and plasma cortisol (ng/mL) for the experimental and control tank over the time course of the experiment (b). The arrow indicates the critical oxygen level where cortisol levels for Atlantic salmon, significantly changed at 4.27 mg/L in seawater.

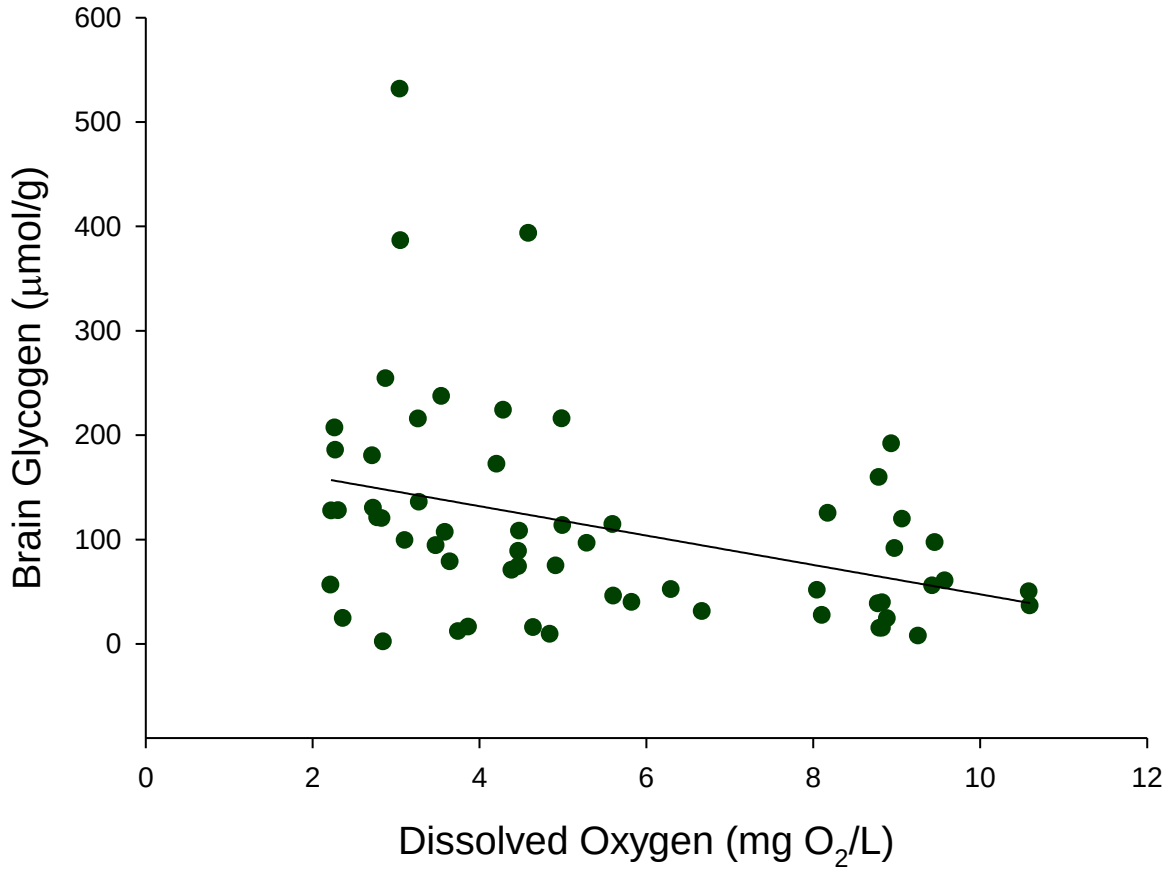

Figure 22. The relationship between brain glycogen (µmol/g) and the dissolved oxygen concentration (mg O_2/L) in seawater for Atlantic salmon can be determined by the equation $Glycogen_{Brain}$ (µmol/g) = -14.068*DO+188.272 (F=8.815, df=57, p=0.004, r^2=0.134).

Experiments # 3 and #6 defined hypoxia in freshwater and seawater and provide interesting results as to where the physiological changes occur along the oxygen gradient. It is clear that each physiological parameter behaves differently along the oxygen gradient as well as differently in freshwater and seawater. Consequently, each provides different information about hypoxia and a hypoxic event. In freshwater, of the parameters measured the first clear physiological change occurs in the abundance of RBC present (HCT). This change in HCT occurred at a DO concentration of 5.37 mg O_2/L (at 14°C is ~52% saturation). The plasma cortisol results showed that it was not until the DO concentration decreased to 3.61 mg O_2/L that fish displayed a clear stress response when cortisol increased rapidly. These

parameters show similar trends in seawater however the physiological changes occur at different DO concentrations. Haematocrit plateaus at a much lower DO concentration of 3.69 mg/L (at 11.6°C is ~42% saturation) indicating that HCT is not as susceptible to hypoxia in seawater. In contrast, plasma cortisol results indicate that newly transferred smolts are at a greater risk of being stressed at much higher oxygen saturation levels. All these critical DO levels are below the thresholds where industry considers hypoxia to begin (D. Mitchell and L. Hubbert, personnel communication). The remainder of the parameters measured in these experiments which showed significant relationships with DO concentrations also showed large individual variation that, unfortunately, prevented a the determination of a clear "break point". This may be due to the individual variation or that these parameters conform to the external environment and therefore the population of fish tested may be considered to be "glycogen conformers" (see above). Regardless of the reason, these data provide excellent baseline information to build upon.

3. Determine the physiological effects of a routine hypoxic event and if it differs between families

Two pedigreed families (five fish each) plus an additional four fish from random families were examined from the 2009 year-class of the SALTAS selective breeding program. These fish had already undergone conditioning for the spawning season and therefore the experiments were conducted on starved fish. All fish that were used in this experiment were ripe males. Fish were acclimated to sea water (> 30‰) over a three week period.

A 12 h period of recovery was chosen based upon the existing literature (Davis and Schreck, 1997; Sadler, et al., 2000b; Mandic, et al., 2008) showing that within a 24 h period a return to starting levels of plasma cortisol and metabolic rate should have occurred. We were interested in both the physiological and biochemical differences which would be evident prior to a complete return to starting levels (i.e. recovery) of parameters and therefore 12 h was chosen.

The only significant difference between families was in white muscle glycogen content ($t= 2.96$, $df= 4$, $p = 0.042$). This result should be viewed with some caution

as there were limited sample sizes for family #8. Three of the five samples taken were unusable and were eliminated from the analysis. There were no other significant differences between families (Table 5).

Of the fish tested, 50% were oxygen regulators and 50% oxygen conformers. The metabolic rate of fish which were oxygen regulating was significantly lower (143.78 ± 23.3 mg O_2/ kg / h) than those conforming (255.59 ± 11.88 mg O_2/ kg / h; F=2.02, df=11, p = 0.002; Fig. 23). This confirms previous observations (Barnes et al., 2011) that at high oxygen levels (>80% saturation) the metabolic rate of oxygen regulators is at least 40% lower than of oxygen conformers. Furthermore, this large difference is maintained over a wide range of DO concentrations. This is indicative of a more metabolically efficient and therefore more robust fish. All fish were measured at 11h after the flow was reinstated for a 1 h metabolic rate check to determine if the fishes metabolic rate had returned to starting levels. All fish had returned to their normal metabolic rate within this time period.

Oxygen conformers had significantly (t= 2.25, df=11, p = 0.046) higher haematocrit (63.74% ± 1.89) than oxygen regulators (55.39% ± 3.02, Fig. 24). There were no other significant differences between regulators and conformers (Table 6).

The cortisol analysis provided an interesting result with three fish still having elevated cortisol levels (>60ng/mL) at the 12 h measurement. These fish would be considered stressed, while a further six fish had cortisol levels that were between 30-60 ng/mL and considered moderately stressed (Trenzado, et al., 2008). The remaining five fish had low cortisol levels (<30 ng/mL) which were in line with the control values from Experiment #6 (below) in seawater and would not be considered stressed (Trenzado, et al., 2008). Whilst there was no relationship between cortisol at 12 h and whether the fish was regulating or conforming, it is apparent that at 12 h we have not yet captured the point where differences may have occurred. We predict that between 12 and 24 h the majority of fish would show a complete recovery as indicated through the literature (van Raaij, et al., 1996; Cnaani and McLean, 2009; Lays, et al., 2009) What is not known is whether fish exposed to this level of hypoxia would show the same response when exposed to a further hypoxic event.

Table 5. Mean values (± standard deviation) for oxygen consumption rates, haematology, plasma L-Lactate and cortisol and tissue glycogen compared between the family lines tested for Atlantic salmon, *Salmo salar* in seawater.

Family	***7***	***8***	***t***	***df***	***p-value***
Oxygen Consumption Rate (mg O_2/kg/h)	252.39 ± 50.92	168.73 ± 68.53	2.19	8	0.060
Haematocrit (%)	62.86 ± 6.96	59.52 ± 6.76	0.77	8	0.464
Haemaglobin (g/dL)	18.36 ± 4.54	15.87 ± 4.42	0.88	8	0.405
MCHC	28.96 ± 5.35	27.20 ± 8.89	0.38	8	0.713
L-Lactate (mM)	22.97 ± 22.55	24.55 ± 18.17	-0.11	6	0.916
Cortisol (ng/mL)	34.60 ± 15.32	40.37 ± 21.84	-0.45	7	0.670
$Glycogen_{WM}$ (µmol/g)	65.27 ± 26.30	6.88 ± 1.77	2.96	4	0.042
$Glycogen_{LIVER}$ (µmol/g)	38.55 ± 9.03	50.11 ± 46.84	-0.42	4	0.696
$Glycogen_{BRAIN}$ (µmol/g)	8.23 ± 9.79	42.26 ± 27.94	-1.59	4	0.187

$Glycogen_{WM}$ is the glycogen content in the white muscle

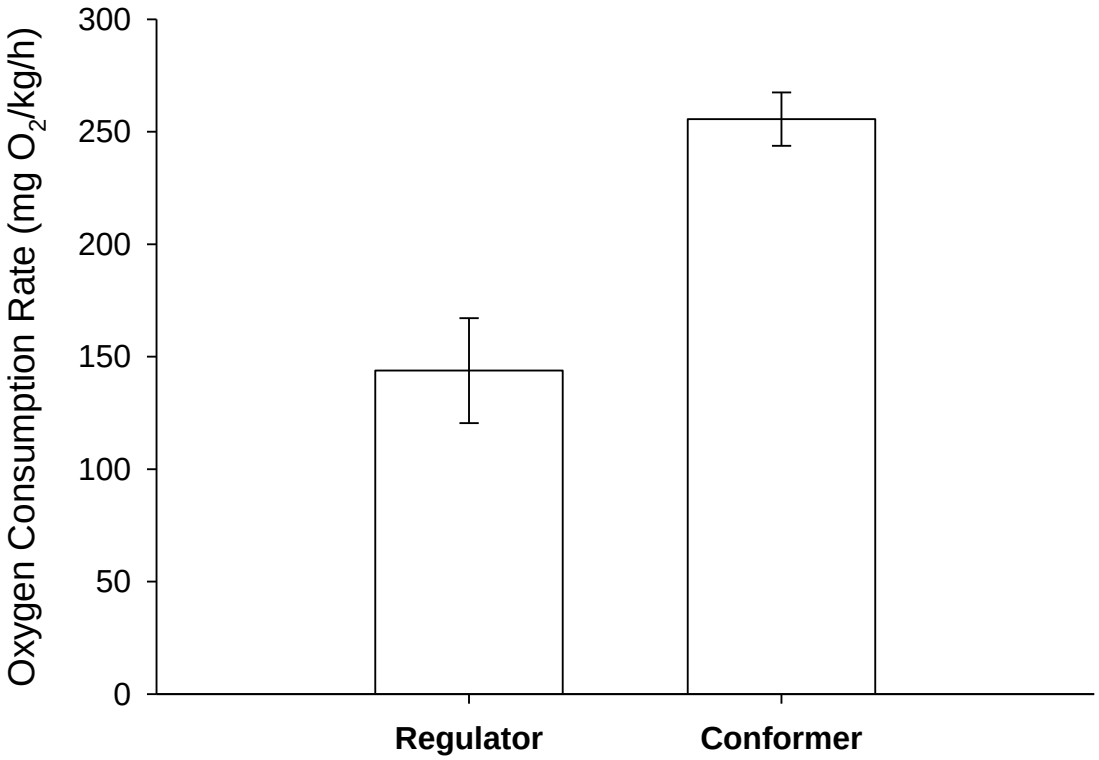

Figure 23. The mean (± standard deviation) oxygen consumption rate (mg O_2/kg/h) at near saturation levels (> 80% saturation) for all regulators and conformers.

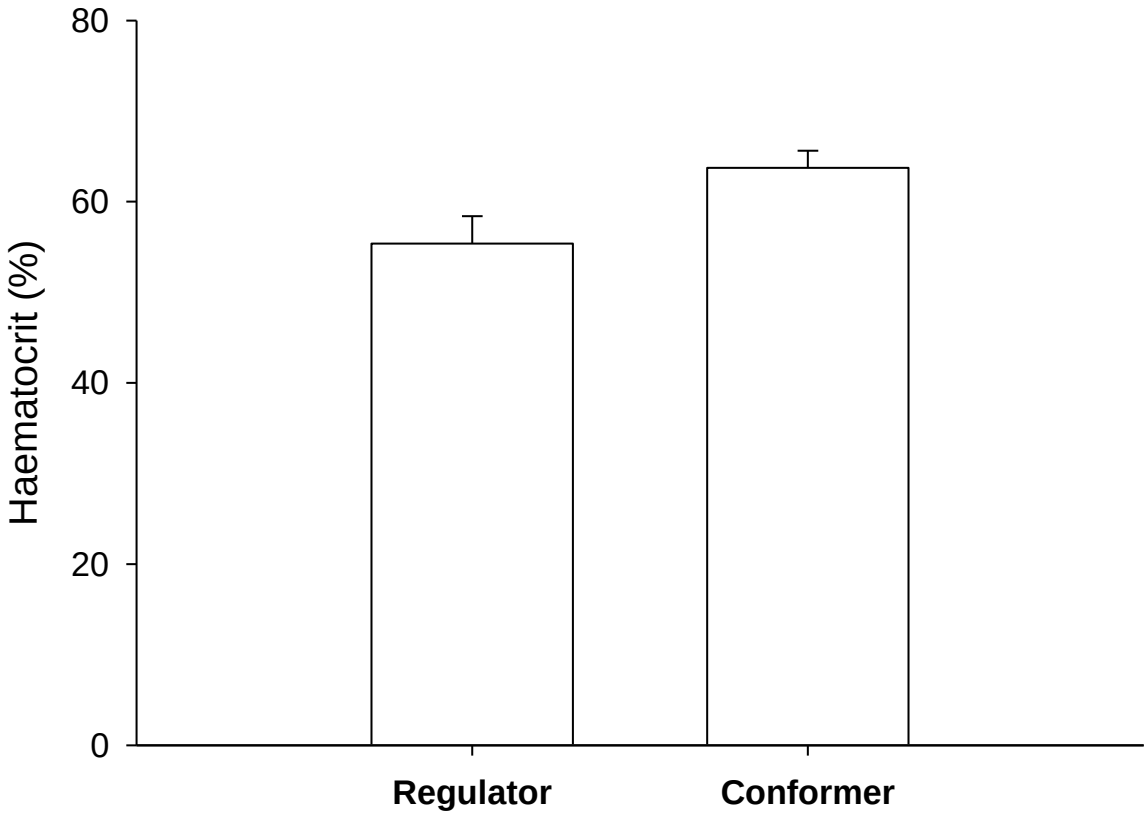

Figure 24. The mean (± standard deviation) of haematocrit (%) for all regulators and conformers.

Table 6. Mean (± standard deviation) for oxygen consumption rates, haematology, plasma L-Lactate and cortisol and tissue glycogen compared between males and females (all families combined) for Atlantic salmon, *Salmo salar* in seawater (Experiment #6).

	O_2 Regulators	***O_2 Conformers***	***t***	***Df***	***p-value***
Oxygen Consumption Rate (mg O_2/kg/hr)	143.78 ± 61.74	255.59 ± 29.12	4.04	11	0.002
Haematocrit (%)	55.39 ± 7.98	63.74 ± 4.63	2.25	11	0.046
Haemaglobin (g/dL)	15.60 ± 4.76	16.62 ± 4.18	0.41	11	0.691
MCHC	27.98 ± 6.50	26.31 ± 7.16	-0.44	11	0.668
L-Lactate (mM)	16.34 ± 11.25	32.34 ± 19.76	1.65	8	0.137
Cortisol (ng/mL)	43.55 ± 11.35	35.95 ± 24.68	-0.72	10	0.486
$Glycogen_{WM}$ (µmol/g)	29.54 ± 46.54	42.59 ± 26.80	0.48	6	0.644
$Glycogen_{LIVER}$ (µmol/g)	50.30 ± 46.72	38.37 ± 9.32	-0.43	4	0.687
$Glycogen_{BRAIN}$ (µmol/g)	22.88 ± 33.06	39.99 ± 21.75	0.83	5	0.442

$Glycogen_{WM}$ is the glycogen content in the white muscle

4. Determine if the ability to regulate is affected by swimming and if it differs between families.

Examination of families was not possible for this objective due to the large size of the fish at the time of pedigree. When individuals became available they were already too large (> 1kg) for the swimming respirometer. Therefore, fish from the general population were utilised for these experiments. The ability to oxygen regulate was assessed in ten fish (168.3 ± 37.5 g) in this experiment. The average swimming speed was 1.33 ± 0.14 BL/s and chosen to represent a flow rate which would be comparable to those found within the industry.

Individual oxygen consumption data clearly showed that all the fish were oxygen regulators (Fig. 25). This was not completely surprising because oxygen regulation during swimming has been shown in other fish, Adriatic sturgeon which had previously all been oxygen conformers all became oxygen regulators when swimming at low speeds and had a lower metabolic rate when forced to swim at 0.5 BL/s (McKenzie, et al., 2007). The mean plateau metabolic rate was 245.83 ± 55.74 mg O_2/ kg / h. Whilst this metabolic rate is approximately 2 times higher than found for oxygen regulators in the earlier experiments of this project, it must be noted that the fish used in this experiment were much smaller (167.34 ± 33.53 g) than in the other experiments (Table 7) and were forced to swim against a constant current which increases the metabolic demands on the fish and therefore the metabolic rate increases accordingly to meet those demands (Brett and Groves, 1979).

The mean critical oxygen threshold (P_{crit}), the point where oxygen regulators were no longer able to regulate their metabolic rate and conform to the environmental oxygen concentration was 4.0 ± 0.5 mg O_2 /L with a range from 3.2 to 4.8 mg O_2/L. This showed some individual differences however P_{crit} was not correlated with the swimming metabolic rate. The duration of the regulation period was 13.93 ± 3.08 h. Average haematocrit for all fish was 39.37 ± 7.38%. The average haemoglobin concentration was 15.45 ± 3.55 g/dL and when the haematocrit and haemoglobin were combined the average MCHC was 39.00 ± 3.91. Average lactate concentrations were 7.32 ± 7.31 mM with values ranging from 1.63 to 16.95 mM. These results show that there is a large amount of variation among swimming

individuals. Lactate above 5 mM suggest these individuals were in an anaerobic state based on the literature for salmonids (Sadler, et al., 2000b; Steinhausen, et al., 2008). However, there were no significant relationships between lactate and swimming speeds, fish weight and condition factor. These relationships were examined to determine if fish size played a factor in the fish entering an anaerobic state. Swimming speed was also examined to determine if the swimming speed was too fast and therefore forced individuals to enter an anaerobic state.

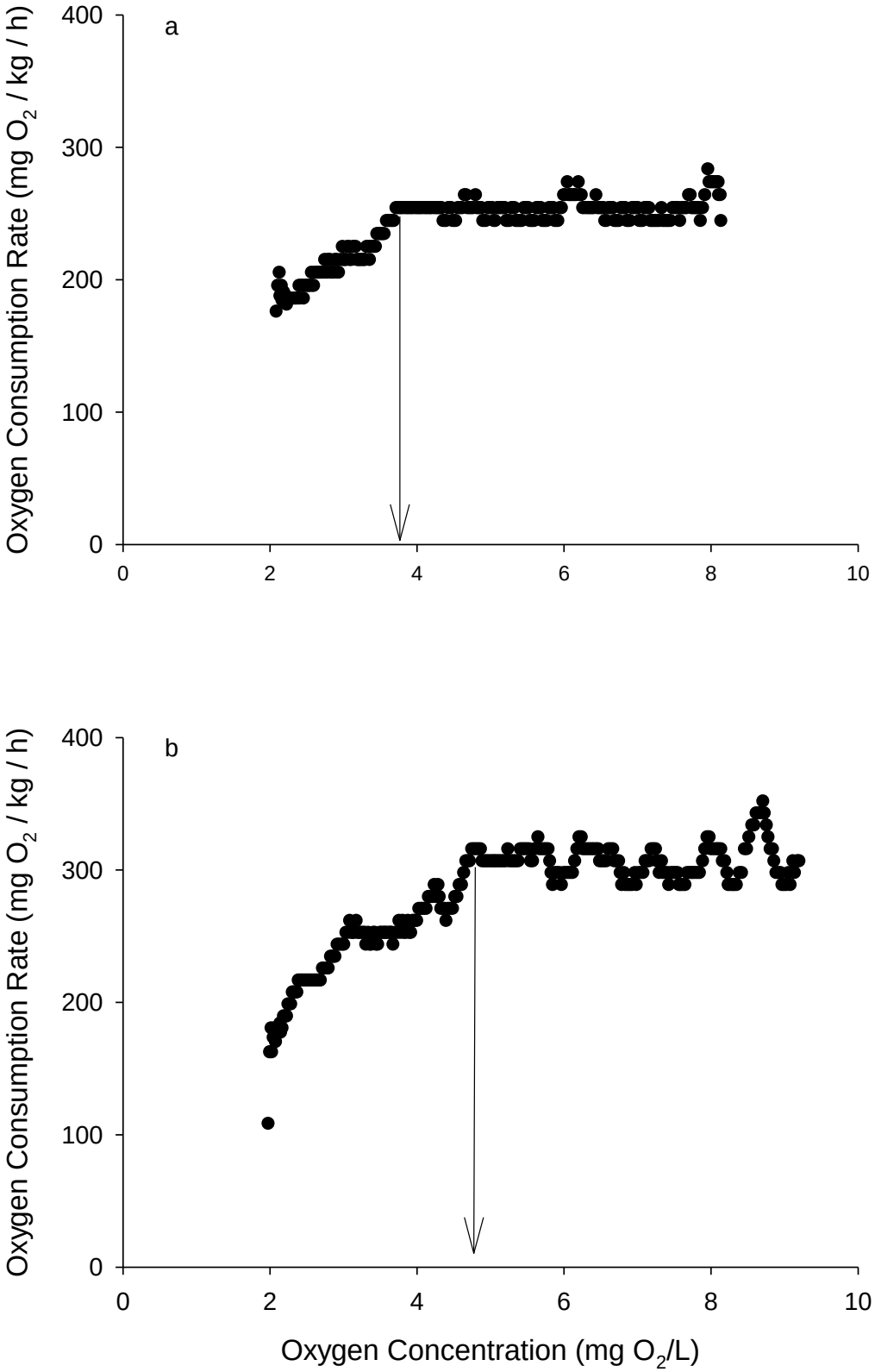

Figure 25. The oxygen consumption rate of two representative fish in the swimming respirometer. The arrow indicates the critical oxygen threshold (P_{crit}, mg O_2/L)for fish (a) is 3.91 mg/L and (b) is 4.76 mg/L.

Benefits and adoption

This research directly benefits the Tasmanian Atlantic salmon industry by showing that oxygen regulation does occur within the population. There are some differences in the ability to oxygen regulate among pedigreed families. Distinct differences occur between male and female fish in the ability to oxygen regulate as well as many biochemical and physiological parameters. This is a critical finding due to the industry's high reliance on all female populations.

The nature of this grant was to explore a wide range of baseline data from a large number of parameters relating to hypoxia and how individual fish cope as they go through mild to severe hypoxic conditions. Individual fish cope with the stress of hypoxia at different levels and this is related to sex but not to families. This was also explored when fish were subjected to a hypoxic event and then recovered for 12 h and differences between individuals returned to various level of stress (based upon cortisol level). Further research is needed to determine the extent of these differences among families and between males and females.

This research was originally designed to examine these parameters at a number of fish sizes and temperatures which span the thermal tolerance of Atlantic salmon with a focus on high temperatures. A large part of the original proposal was to focus on determining if the ability to oxygen regulate was inherent or acquired, this was lost as a result of industry-driven modifications to the original proposal.

Further development

Results of this project have been circulated throughout industry by way of milestone reports and the Australiasian Aquaculture 2010 meeting. Final results of this project will be presented at the TSGA research and development meeting on November 30th, 2011 and at the Australasian Aquaculture 2012 conference in May, 2012. Copies of this report will also be distributed to the Tasmanian salmon industry.

Further research is needed to:

- Determine the extent of family differences
- Further explore differences between males and females
- Investigate how oxygen regulation effects fish at high temperatures (i.e. summer conditions)
- Clarify if oxygen regulation is an inherent or learned ability and whether preconditioning to hypoxia (or any stressor) increases tolerance and/or decreases time to recovery
- Establish the ability of, and time required for, individuals from different families to recover from mild to extreme hypoxic conditions over the thermal tolerance range (especially at high temperatures)

These topics will be addressed further through a PhD research project potentially starting February 2012. Results will be disseminated to the industry as they become available.

Planned outcomes

One of the main aims of this project was to confirm some preliminary results that oxygen regulation did occur within the Tasmanian Atlantic salmon population. We have clearly shown that oxygen regulation does occur.

This information will be published in peer-reviewed journals and presented at the Australiasian Aquaculture meeting 2012 in Melbourne, Australia and at any preceding industry meetings.

Conclusions

This project confirmed that oxygen regulation does occur in the Tasmanian population of Atlantic salmon. Between 30% and 64% of fish in the initial three families tested were able to oxygen regulate. Fish which have the ability to oxygen regulate have a metabolic rate that is approximately 40% lower than fish which are oxygen conformers. These results are interesting because the reduced metabolic rates may firstly relate to the overall robustness of the fish and secondly to the overall efficiency of the individual fish that results in more efficiency growth. Female

fish have higher metabolic rates, higher critical oxygen thresholds, higher stress and lactate levels than male fish from the same families.

A clear stress response from the progressive hypoxia in freshwater was not evident until the DO concentration reached 3.61 mg O_2/L. However, physiological changes occurred at higher DO levels with haematocrit increasing until the DO concentration reached 5.37 mg O_2/L, at this point the haematocrit reached a plateau. The same pattern was seen in fish which had recently been transferred to seawater, however these fish were clearly stressed at higher DO concentrations (4.27 mg O_2/L). This suggests fish recently transferred to seawater would be more sensitive to decreased DO. Haematocrit levels did not reach a plateau until the DO concentration reached 3.69 mg O_2/L in seawater.

To compare metabolic rates across experiments for individual fish (Table 7) the mean metabolic rate was weight scaled to 0.75 (Kleiber, 1961; Houlihan, et al., 1995) in order to allow direct comparison between fish of different sizes. From this scaled data, it is clear that individual regulators were not different between experiments until Experiment #7 where the scaled metabolic rate is at least 50% greater than in the other experiments. In Experiment #7 we examined individual fish experiencing a progressive hypoxia under constant flow conditions similar to those they may encounter on farm. The metabolic rate of swimming fish was higher, swimming requires energy expenditure and adds another component to the energy balance of fish especially under stressful conditions (i.e. hypoxia). Despite the higher metabolic rate there was no difference in the critical oxygen threshold (P_{crit}) values indicating that fish under flow conditions are able to oxygen regulate down to similar DO concentrations as fish under static conditions.

It is clear from this project that a great deal more research is needed to properly examine the full extent of family differences by examining greater numbers of fish from a greater number of families as well as determining differences between males and females from each pedigreed family. It will be critical to examine differences between individuals at high (i.e. summer) temperatures as the likelihood of hypoxia is increased as temperature increases. To determine if the ability to oxygen

regulate is genetic or a learned ability, additional research is needed to establish whether preconditioning to hypoxia (or any stressor) increases tolerance and/or decreases time to recovery.

Table 7. Summary of experiments where fish individual fish experienced a progressive hypoxia. Mean fish weight (g), temperature (°C), salinity (‰), metabolic rate (mg O_2/kg/h) and weight scaled metabolic rate (mg O_2/kg $^{0.75}$/h) of oxygen regulator and oxygen conformers, Pcrit (mg O_2/L) and duration (h) of the oxygen regulation period are presented.

	Fish Weight	**Temperature**	**Salinity**	**VO_2 (mg O_2/kg/h)**		**Weight Scaling (mg O_2/kg$^{0.75}$/h)**		**Pcrit**	**Duration**
	(g)	**(°C)**	**(‰)**	**O_2 Regulators**	**O_2 Conformers**	**O_2 Regulators**	**O_2 Conformers**	**(mg O_2/L)**	**(h)**
Experiment #									
2	1504.4 ± 409.9	11.97 ± 0.62	0	107.43 ± 20.60	178.85 ± 36.64	33.37	48.91	3.31 ± 1.18	11.92 ± 5.21
4	1498.6 ± 337.4	11.69 ± 0.21	>30	143.78 ± 61.74	255.59 ± 29.12	41.52	63.92	4.09 ± 0.12	6.18 ± 2.83
5	1245.1 ± 163.7	11.53 ± 1.11	>30	136.16± 30.05	169.12 ± 38.68	39.86	46.90	3.35 ± 0.83	8.44 ± 5.05
7	167.3 ± 33.5	13.53 ± 1.04	>30	245.83 ± 55.74	--	62.08	--	3.97 ± 0.50	14.21 ± 3.57

Metabolic rate is also scaled to 0.75 to remove the fish size component of metabolic rate and permit a direct comparison between fish of different sizes (Kleiber, 1961; Houlihan, et al., 1995).

Experiments 3 and 6 are not included because individual fish were not examined in those experiments

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Appendix 1 Intellectual Property

The intellectual property and valuable information arising from this report are:
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Appendix 3

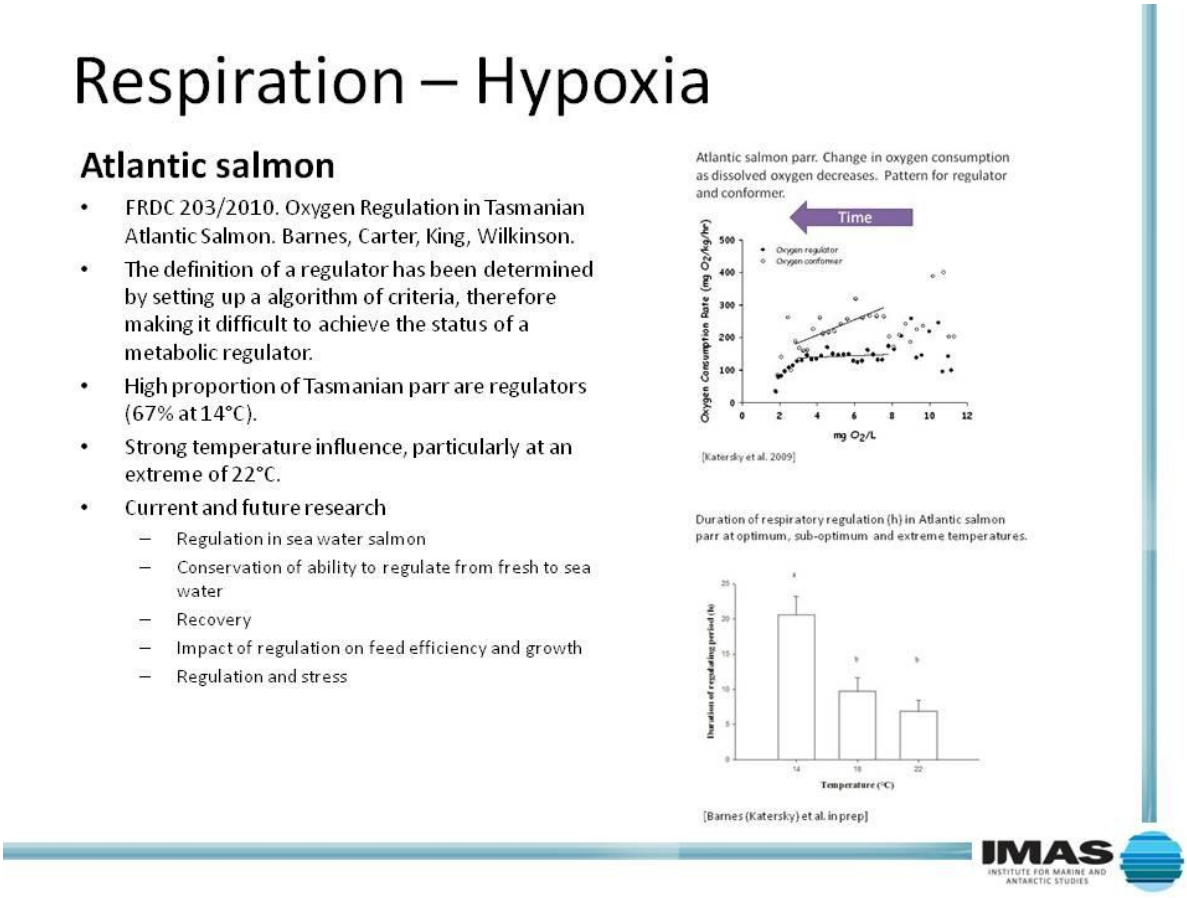
Published manuscripts

Barnes, R.K., King, H., Carter, C.G., 2011. Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population. Aquaculture 318, 397-401.

Conference presentations

Purser, J., Battaglene, S., Carter, C., Hobday, A., Katersky, R., Lyne, V., Nowak, B., Pinkiewicz, T., Wilkinson, R. 2011. Challenges in the Tasmanian salmon industry with a focus on climate change. *COST 867: International Workshop on Fish Welfare*, 8-10 February, Madrid, Spain.

Carter, C.G. 2011. Nutritional Physiology: the ins and outs of nutrients. IMAS Fisheries, Aquaculture and Coasts Research Overview, 2nd May, Hobart.



Media release

MEDIA RELEASE

DATE: FRIDAY MARCH 5, 2010
ATTENTION: Chiefs of Staff, News Directors

Funding for Atlantic Salmon Research

Funding of $150,000 for new research into the metabolic regulation of Atlantic Salmon has been allocated to the University of Tasmania.

The research team will be led by Dr Robin Katersky of the National Centre for Marine Conservation and Resource Sustainability at the Australian Maritime College, a specialist institute of the University of Tasmania, working with Dr Harry King of Salmon Enterprises of Tasmania, and Prof. Chris Carter of the Tasmanian Aquaculture and Fisheries Institute. They will focus on whether Atlantic salmon are able to regulate their metabolic rate, specifically examining changes after fish transfer to the sea, and any physiological effects associated with exposure to hypoxia (low oxygen) and metabolic regulation.

The University of Tasmania received the funding through the Atlantic Salmon Subprogram of the Fisheries Research and Development Corporation.

Metabolic regulation is when salmon can regulate their oxygen consumption rate when exposed to lower levels of oxygen in the water, which occur as the water temperature increases. The research team hopes to identify fish which can adjust to sea more efficiently and could possible contribute to the selective breeding program.

Dr Katersky said she was really excited to be working directly with industry as part of their strategy to address the possible impacts of climate change This project further develops important applied research lead by Dr Katersky as part of her 2008 Australian Agriculture Industries Young Innovators and Scientist Award where oxygen regulation was shown to occur at suboptimal temperatures in Tasmania.

Atlantic Salmon Subprogram: Oxygen regulation in Tasmanian Atlantic salmon is supported by funding from the Fisheries Research and Development Corporation on behalf of the Australian Government.

Information released by:
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