AN INVESTIGATION INTO THE GENETIC BASIS OF VARIATION IN HYPOXIA TOLERANCE IN ATLANTIC SALMON

by

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Abstract

Episodes of hypoxia are becoming more common along the British Columbia (BC) coast especially in the late summer. When dissolved oxygen drops below optimum levels, fish survival, growth and reproduction are affected; moreover, hypoxia can be lethal to fish, resulting in economic losses to salmon farmers. As a first step towards addressing this challenge for BC salmon farmers, the objectives of this study were to characterize variation in hypoxia tolerance in Atlantic salmon (*Salmo salar*) under culture conditions and identify the genetic basis of this variation in the strains of salmon used by the aquaculture producer Marine Harvest Canada. Using time-to-loss-of-equilibrium (LOE) following exposure to acute hypoxia (2.1 mg/L) as an index of hypoxia tolerance, I show that there are significant differences in hypoxia tolerance within and between the strains of Atlantic salmon examined. For adults in seawater, time-to-LOE at 2.1 mg/L DO ranged from 4.6 min to 126.9 min, and the McConnell strain had better hypoxia tolerance than the Mowi strain. A similar pattern was observed for smolts in freshwater, with time-to-LOE ranging from 4.5 min before 2.1 mg/L DO was reached to 355.4 min at 2.1 mg/L DO. Genotyping-by-sequencing (GBS) was used to identify single-nucleotide polymorphism (SNP) markers in these strains for use in a genome-wide association study (GWAS). GWAS in adult fish in seawater revealed two SNPs associated with hypoxia tolerance using genome-wide FDR correction, and six SNPs associated with hypoxia tolerance using chromosome-wide FDR correction. In contrast, GWAS in smolts in freshwater identified one SNP using genome-wide FDR correction and one SNP using chromosome-wide FDR correction. There was no overlap in the SNPs identified as associated with hypoxia tolerance at these two life stages. In addition, I identified four significant SNPs associated with body mass in adults with chromosome-wide FDR correction and two SNPs associated with body mass with genome-wide FDR correction and

fifty-eight SNPs associated with body mass with chromosome-wide FDR correction. These findings provide promise for follow-up work on SNP markers that could potentially be used for marker-assisted selection to improve hypoxia tolerance and growth in Atlantic salmon.

Lay Summary

Aquaculture is a very important industry in British Columbia, with the farming of Atlantic salmon contributing more than $475 million annually to the BC economy. Low levels of dissolved oxygen in water (hypoxia) are becoming more common along the BC coast. Hypoxia can be lethal to fish, resulting in economic losses to salmon farmers. The goal of this study was to identify genetic markers that can be used to improve the ability of salmon to tolerate low oxygen conditions in sea cages. I identified a total of ten genetic markers associated with hypoxia tolerance either in adults (seawater) or in smolts (freshwater). There was no overlap in the markers identified as associated with hypoxia tolerance at these two life stages. These findings overall provide promising genetic markers for follow-up work that could potentially be used for artificial selection to improve hypoxia tolerance in Atlantic salmon.

Preface

Chapter 2 and Chapter 3 of this thesis were performed in collaboration with Dr. T. M. Healy, Dr. A. P. Farrell, Y. E. Sheehan, Dr. D. Morrison and Dr. P. M. Schulte. I collected hypoxia data and performed all laboratory procedures. Genotyping by sequencing and SNP calling were conducted in the University of Cornell Genomic Diversity Facility. Dr. T. M. Healy performed paralog and SNPs filtering. I ran GWAS analysis and completed the write up.

I received editorial feedback from Dr. P. M. Schulte, Dr. A. P. Farrell, Dr. T. M. Healy and Dr. R. H. Devlin.

The experiments in this thesis followed the protocols that were in accordance with the UBC Animal Care Committee; protocol number: A15-0172.

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List of Abbreviations

ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BC	British Columbia
°C	degrees Celsius
DNA	deoxyribonucleic acid
e.g.	for example
FAO	Food and Agriculture Organization of the United Nations
FDR	false discovery rate
g	grams
GWAS	genome-wide association study
Hb	hemoglobin
L	litre
mg	milligram
min	minute
ng	nanogram
NKAα1b	Na^+/K^+-ATPase α-1b isoform
µg	microgram
PCR	polymerase chain reaction
Q_{10}	temperature quotient
r^2	coefficient of determination
RNA	ribonucleic acid
SNP	single nucleotide polymorphism

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Chapter 1. General Introduction

1.1 The importance of aquaculture

The production of global capture fisheries has remained at around 90 million tonnes since the late 1980s, whereas aquaculture has shown a strong and continual annual growth rate of 6.1%, from 36.8 million tonnes in 2002 to 73.8 million tonnes in 2014 (FAO, 2016, 2012). In 2014, aquaculture reached a milestone where the human consumption of farmed species exceeded that of global capture fisheries for the first time in history (as has been predicted earlier (Naylor et al., 2000)), increasing from just 7% of total fish supply in 1974 (FAO, 2016). Furthermore, the World Bank projects that aquaculture will continue to expand and fill the demand for seafood such that by 2030 approximately 62% of seafood for human consumption will be produced by aquaculture (Msangi et al., 2013).

Farmed Atlantic salmon (*Salmo salar*) is one of the most economically important finfish species in global aquaculture. Global production of farmed Atlantic salmon has increased 10-fold from around 225,000 tonnes in 1990 to approximately 2,300,000 tonnes in 2014 (FAO, 2016). Today, Atlantic salmon is the most cultured fish species in western countries and the most cultivated salmonid globally (FAO, 2012). The majority of farmed Atlantic salmon are produced from Norway, Chile, Scotland and Canada, with an estimated value of more than $10 billion worldwide in 2014 (Marine Harvest, 2016; Seafish, 2012). As a result, Atlantic salmon aquaculture is very important both economically and socially.

Understanding potential factors that can limit Atlantic salmon aquaculture production and developing approaches that could be employed to counter these limitations are becoming necessary. One such factor is the dissolved oxygen (DO) level in water, which can impact fish

development (Burt et al., 2012; vanRaaij et al., 1996) and survival. Therefore, my thesis explores the basis of individual and familial variation in tolerance to environmental hypoxia (low oxygen in water).

1.2 Hypoxia has already become a challenge in aquaculture, particularly in marine sea cages

Oxygen is essential for all living organisms that rely on aerobic respiration to produce cellular energy (Diaz & Breitburg, 2009). However, water contains 20-40 times less oxygen than air primarily due to the low solubility of oxygen in water. This, coupled with a high oxygen usage by organisms and poor exchange with the atmosphere, leads to frequent low DO in a broad range of aquatic environments (Diaz & Breitburg, 2009). Low DO conditions occur when the rate of oxygen consumption by organisms exceeds that of resupply (Diaz & Breitburg, 2009), or when there is water turnover from anoxic zones to normoxic zones, such as when upwelling induces the transportation of deep, nutrient-rich, anoxic water into the sea surface (Grantham et al., 2004).

Severe hypoxic events in coastal areas have caused many massive fish mortality events all over the world; moreover, some hypoxia-sensitive species have been removed in aquatic systems due to hypoxia both in the short-term and long-term (Diaz & Rosenberg, 1995; Wu, 1982). Hypoxia in an aquaculture setting has also led to massive fish mortality (Azanza et al., 2005; Bouchet et al., 2007; Grantham et al., 2004). For example, at a milkfish (*Chanos chanos*) farm in the northern Philippines, massive mortality occurred when the level of DO dropped to 2.1 mg/L, and this hypoxic incident resulted in $120,000 loss to the milkfish farming industry (Azanza et al., 2005). In Tasmania, a severe hypoxic event in aquaculture sea cages led to an

unprecedented loss of 85,000 farmed salmon adults (Blucher, 2015). A recent study has reported that a disastrous mass mortality of Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) occurred in Lake Hashenge, Tigray when the DO levels fell to 2.39 mg/L (Teame et al., 2016).

In a marine cage environment, the DO levels can fluctuate substantially with depth and over time (Burt et al., 2012; Johansson et al., 2006; Johansson et al., 2007; Oppedal et al., 2011). Several studies have shown that DO levels in sea cages may drop to 30% of air saturation, especially in late summer and fall with high water temperature and high stocking density (Johansson et al., 2006; Oppedal et al., 2011). In general, algal photosynthesis and diffusion of atmospheric oxygen are the main supplies of new oxygen to the water in a marine fish farm (Davis, 1975); however, the amount of oxygen produced by photosynthesis within a marine fish farm is often insufficient to meet the oxygen demand of the fish biomass (Wildish et al., 1993). Therefore, oxygen requirements must depend on physical transport (water currents), which is driven by different components such as wind conditions, tidal movements and freshwater runoff (Wildish et al., 1993). Previous studies also have shown that the physical transport of oxygen is limited by many factors such as mesh size of net pens, cage arrangement, net fouling, local topography and the existence of a pycnocline (Inoue, 1972; Johansson et al., 2006).

1.3 Rise of eutrophication and climate change drives hypoxia

1.3.1 Eutrophication drives hypoxia

In addition to hypoxic conditions generated as a result of standard aquaculture practices, widespread eutrophication due to anthropogenic activities has increased the prevalence and severity of hypoxia in many aquatic systems (Diaz, 2001). Eutrophication occurs when the

production of organic matter exceeds the capacity of an ecosystem to process it (Nixon, 1995), and is primarily driven by excess nutrient enrichment on coastal ecosystems (Diaz & Breitburg, 2009). Nitrogen is usually the limiting nutrient in marine systems (Boesch, 2002), and rise of anthropogenic nitrogen from the activities such as agriculture and the release of raw sewage have led to dramatic increase in algae production (Diaz & Rosenberg, 1995; Ficke et al., 2007). With an increase in algae production, aerobic respiration by algae increased, leading to a decrease in DO in the water, particularly at night when photosynthesis cannot occur (Diaz & Rosenberg, 1995; Ficke et al., 2007). Eutrophication can also lead to the reduction of DO levels in bottom water due to the accumulation of dead algae and organic matter on the seabed which accelerates microbial respiration (Rabalais et al., 2002). The more organic matter introduced into the marine coastal ecosystems, the more oxygen is required by marine bacteria to metabolize the organic material (Diaz & Breitburg, 2009).

Furthermore, the build-up of unconsumed feed and fecal wastes under a cage site can stimulate microbial production, which leads to a local anoxic zone. The changes in ocean currents can upwell the deep hypoxic water, resulting in hypoxia in sea cages.

1.3.2 Global warming decreases dissolved oxygen

Many models of earth's climate such as the global circulation models (GCMs) used by the Intergovernmental Panel on Climate Change (IPCC) have predicted that the mean global temperature will increase by 1 to 7 °C over the next hundred years (Ficke et al., 2007; Houghton et al., 2001). In water, the solubility of a gas decreases as temperature increases, and thus an increase of water temperature will reduce the availability of oxygen to aquatic organisms (Weiss, 1970). The physiological temperature for most fish ranges from 0 to 40 °C, and within this

temperature range, DO levels decrease by 10 to 20% for every 10 °C increase in water temperature (Farrell & Richards, 2009). Over the last 100 years, seawater temperatures have increased nearly 1 °C in many tropical aquatic ecosystems, and are predicted to increase at a rate of approximately 1 to 2 °C per century (Hoegh-Guldberg, 1999). Thus, if sea temperature increases by 2 °C over the next hundred years, the DO levels may decrease by 2 to 4%. Furthermore, a recent study has shown that the dissolved oxygen level in the global ocean has declined more than 2% over the last 50 years, and could decrease 1 to 7% by 2100 based on ocean models (Schmidtko et al., 2017).

1.3.3 Global warming increases metabolism

Temperature has a significant impact on a fish's oxygen demands (Farrell & Richards, 2009). As the temperature increases, the metabolic demand for oxygen in cold-blood organisms (e.g. fish, crabs and bivalves) increases (Brown et al., 2004). It has been previously reported that Q_{10} values are usually around 2 for O_2 consumption (Farrell & Richards, 2009). Based on the prediction by Hoegh-Guldberg (1999) that seawater temperature may increase by 2 °C over the next hundred years, oxygen demands for cold-blood aquatic organisms could potentially increase by 20%, which could decrease hypoxia tolerance (Vaquer‐Sunyer & Duarte, 2011). Indeed, hypoxia tolerance in Chinese bream (*Parabramis pekinensis*) decreased significantly with an increase of temperature (He et al., 2015). Nilsson et al. (2010) demonstrated that hypoxia tolerance in Doederlein's cardinalfish (*Ostorhinchus doederleini*) and lemon damselfish (*Pomacentrus moluccensis*) decreased significantly when exposed to a 3 °C rise in rearing water temperature. Therefore, global warming will likely make marine ecosystems more vulnerable to becoming hypoxic and can speed up the development of hypoxia in coastal areas.

1.4 Consequences of hypoxia for fish

During periods of hypoxia, fish face the challenge of a reduced aerobic ATP production through oxidative phosphorylation. Physiological functions in fish are compromised at the water PO_2 levels where the arterial blood O_2 content starts to decrease (Farrell & Richards, 2009). At these water PO_2 levels, the ability of the fish to extract oxygen from the environment is reduced, and the fish experiences hypoxemia (Farrell & Richards, 2009). Routine O_2 demand may still be sustained through compensatory responses (e.g. increasing gill ventilation and perfusion, increasing cardiac output) (Farrell & Richards, 2009; Remen et al., 2013), but functional activities such as locomotion, reproduction and growth, are impacted (Farrell & Richards, 2009). If the water PO_2 continues decreasing, a point will be reached where such compensatory responses become unable to maintain the routine metabolic activities. This water PO_2 is known as the critical oxygen tension (P_{crit}), the minimum oxygen level required to maintain the standard metabolic rate (He et al., 2015).

At water PO_2 levels below the P_{crit}, a fish cannot maintain oxidative ATP production that is sufficient for basic needs and anaerobic metabolism increases to compensate for deficiencies of energy production from aerobic respiration (Remen et al., 2012; Vianen et al., 2001). Although ATP can be generated in the absence of oxygen, anaerobic energy production is much less efficient than aerobic energy production (Richards, 2009), generating approximately 1/15-1/30 ATP per mole of substrate consumed when compared to aerobic respiration (Richards, 2009). Fish may be able to increase anaerobic ATP production to maintain their routine metabolic rate in the short-term by upregulating anaerobic metabolism; however, this leads not only rapid depletion of fermentable substrate (e.g. glycogen), it also results in the accumulation of wastes that have negative consequences.

A deficient O_2 supply due to hypoxia has negative impacts on fish survival, growth and reproduction. Hypoxia can lead to a decrease in fish growth, primarily due to loss of appetite (Davis, 1975; Wang et al., 2009). An early study has showed that the growth rates of juvenile rainbow trout (*Oncorhynus mykiss*) decreased when the DO levels dropped below 7.0 mg/L at 15 °C, and the fish fed much less when the DO levels fell below 6.0 mg/L at 15 °C (Pedersen, 1987). When juvenile turbot (*Scophthalmus maximus*) and European sea bass (*Dicentrarchus labrax*) were exposed to hypoxia (3.2 mg/L DO) for 42 days, the feed intake was 1.7-1.8 times lower in turbot and 1.5-1.7 times lower in sea bass than in the fishes raised in normoxic conditions (7.4 mg/L DO) (Pichavant et al., 2001). Atlantic salmon fed to satiation and reared in saltwater at 16 °C demonstrate a decrease of appetite when the DO level fell to just 70% of air saturation; skin lesions increased at around 60% of air saturation; feed conversion and growth were reduced at around 50% of air saturation; and impaired osmoregulation and mortalities occurred at around 40% of air saturation (Oppedal et al., 2011). Hypoxia can also impair reproduction and development by preventing testicular and ovarian development, influencing the production and quality of gametes, increasing the chance of failure in fertilization and hatching, and affecting the survival of larva and juveniles (Wu, 2009). When female killifish (*Fundulus grandis*) were exposed to hypoxic conditions (1.34 mg/L DO) for a month, their spawning time was significantly delayed (Landry et al., 2007). Chinook salmon (*Oncorhynchus tshawytscha*) embryos have shown a 6-10 day delay in hatching when they were exposed to hypoxia (4 mg/L DO) (Geist et al., 2006). Another study has shown that hypoxia caused a delay in embryonic development in Rainbow trout and Atlantic salmon which resulted in an increase in mortality and the number of early hatching embryos through secreting excess chorionase (Hamor & Garside, 1976).

However, the mechanisms that cause hypoxic death in fish are poorly understood. Richards (2009) has demonstrated that hypoxia-induced death is related to depletion of substrate, accumulation of toxic substances (lactate and protons) and cellular necrosis. Boutillier and St-Pierre (2000) proposed that a hypoxia-induced succession of events results in cellular necrosis. When hypoxia-sensitive fish are exposed to severe hypoxia, they are unable to produce sufficient ATP to maintain essential cellular functions such as protein synthesis, ion regulation and other metabolic processes (Boutilier & St-Pierre, 2000). Nevertheless, cellular ion regulation plays an important role in cellular survival, and failure of ion regulation can lead to depolarization of plasma and organelle membranes, Ca^{2+} accumulation in the cytosol, the activation of phospholipases and Ca^{2+}-dependent proteases and the rupture of membrane, leading to necrotic cell death (Boutilier & St-Pierre, 2000). Neural tissue (e.g. brain) has the highest turnover rate of ATP. Fish brain has approximately 10-times faster ATP turnover rate than the average body tissue, which suggests that the brain is extremely sensitive to hypoxia (Nilsson, 1996) and thus will initially experience a reduction of ATP levels during hypoxia (Lutz et al., 2003). Hansen (1985) has demonstrated that hypoxia not only can make the brain electrically silent, but also lead to irreversible damage.

1.5 Fish responses to hypoxia

1.5.1 Enhance oxygen extraction

Fish have many strategies to enhance oxygen extraction from hypoxic environments to their tissues. Behaviourally, fish may simply avoid hypoxic environments by swimming away (Chapman & McKenzie, 2009). However, this is not an option for farmed species since they could not escape from the rearing conditions. These fish may acquire more oxygen through

aquatic surface respiration (ASR), which allows them to obtain the relatively higher DO from the air-water interface (Kramer & McClure, 1982). Some fish species even are able to acquire oxygen directly from air through air-breathing (Chapman & Mckenzie, 2009). These fish can gulp air at the water surface and store the air in an air-breathing organ such as modified swimbladders, branchial chambers and guts (Graham, 1997).

Physiological strategies to enhance oxygen uptake include increasing gill ventilation and perfusion, increasing cardiac output, increasing blood oxygen-carrying capacity and hemoglobin (Hb)-O_2 binding affinity (Farrell & Richards, 2009; Nilsson, 2007; Wells, 2009). The ventilatory response to hypoxia, also known as gill hyperventilation, is initiated when oxygen chemoreceptors sense changes of PO_2 in water or/and blood (Perry et al., 2009). Gill hyperventilation to enhance oxygen uptake can be achieved by adjusting the frequency and volume of buccal pumping (Nilsson, 2007). During hyperventilation, an increase in water flow over the gill reduces the difference between inspired PO_2 and expired PO_2, which increases the mean blood-to-water PO_2 gradient; as a result, arterial PO_2 increases. Thus, gill hyperventilation serves either to raise arterial PO_2 or minimize the degree of reduction in arterial PO_2, which is the inevitable outcome of continually dropping water PO_2 (Holeton & Randall, 1967; Wood & Johansen, 1973).

The cardiovascular responses of fishes are also very important for enhancing oxygen uptake from the hypoxic environments and transporting oxygen to the tissues (Gamperl & Driedzic, 2009). The usual cardiac response of fishes to hypoxic conditions is a reflex decrease in heart rate, also known as hypoxic bradycardia (Farrell, 2007). During hypoxic bradycardia, cardiac output can be maintained by the fish heart through a large increase of cardiac stroke volume (V_{SH}) (Farrell, 2007). Hypoxic bradycardia could provide several direct benefits to the

fish heart, mostly because the oxygen supply to the spongy myocardium is insecure and primarily relies on the PO_2 in the venous blood (Farrell, 2007). These benefits include: (1) enhanced oxygen diffusion in myocardium as a result of an increase in the diastolic residence time of blood in the lumen of the heart (i.e., extend oxygen diffusion in myocardium), and a reduction of diffusion distance for oxygen through stretching the cardiac chambers (i.e., increase stroke volume); (2) enhanced cardiac contractility via the negative force-frequency effect; (3) reduced cardiac oxygen demand through a reduced rate of ventricular pressure development (dP/dt); and (4) an increase in coronary blood flow because of an extended diastolic period, which results in decreasing the dependence on PO_2 in venous blood (Farrell, 2007).

Fish also can respond to hypoxia through the adjustments of blood oxygen-carrying capacity and Hb-O_2 binding affinity (Wells, 2009). Exposure to hypoxia can lead to an increase in Hb concentration through the release of erythrocytes from the spleen (Wells et al., 1989). Exposure to hypoxia also can trigger the release of catecholamines into the circulation (Gamperl et al., 1994; Lowe & Wells, 1996; Randall & Ferry, 1992), where the erythrocyte surface receptors bind adrenaline and noradrenaline (Nikinmaa & Huestis, 1984). In teleosts, β_3-adrenergic receptors can increase the pH of the erythrocytes through the regulation of Na^+/H^+ pumps causing an increase of Hb-O_2 affinity (Nikinmaa, 2002). Hb-Oxygen affinity also can be modulated via changing the concentration of allosteric modulators, such as organic phosphates, chloride ions and water (Wells, 2009). The erythrocyte organic phosphates are thought to play a particularly important role in hypoxia, and can bind to specific locations in the central cavity of the Hb tetramer and stabilize the structure in low-affinity conformation (Wells, 2009). Under severe hypoxia, aerobic metabolism will eventually shift to anaerobic metabolism with

accompanying acidosis; this shift will lead to a reduction of ATP and GTP, which can result in an increase of blood-oxygen affinity due to less ATP and GTP binding to Hb (Wells, 2009).

1.5.2 Metabolic rate suppression

At oxygen levels below the point where the behavioural and physiological strategies to obtain oxygen uptake are effective to maintain routine metabolic activities, the fundamental challenge for fishes is metabolic energy balance (Richards, 2009). The ability to suppress metabolic demand to match the limited capacity for oxygen-independent energy production has become a very important strategy for hypoxia survival (Hochachka et al., 1996). Reducing metabolic rate in response to hypoxia has been reported in a range of specialized fish species (e.g., crucian carp (*Carassius carassius*), goldfish (*Carassius auratus*), tilapia (*Oreochromis mossambicus*), rainbow trout) (Richards, 2009). For example, around a 70% reduction in metabolic rate during hypoxia exposure has been reported in hypoxia-tolerant fish, which includes goldfish (Waversveld et al., 1989), tilapia (Ginneken Van et al., 1999) and European eel (*Anquilla anquilla*) (van Ginneken et al., 2001). Metabolic rate suppression can be achieved through controlling or arrest of many processes that are involved in protein synthesis (Lewis et al., 2007; Wieser & Krumschnabel, 2001), membrane ion pumping activity (Richards et al., 2007), RNA transcription, gluconeogenesis, urea synthesis, and other pathways for anaerobic metabolisms (Hochachka et al., 1996). In the goldfish, Jibb & Richards (2008) demonstrated a significant drop of around 70% in liver protein translation rate in response to hypoxia exposure (less than 0.5% air saturation), and these drops were mediated by stopping protein elongation during the process of translation. In the crucian carp, anoxia exposure led to significant decreases in protein synthesis in liver, heart and muscle; these decreases were partially mediated by

decreasing RNA transcription rates (Smith et al., 1996). In the Amazonian oscar (*Astronotus ocellatus*), hypoxia exposure (10% air saturation) caused a rapid decrease in protein synthesis rates across different tissues, such as 27% and 60% decrease of protein synthesis in brain and heart respectively (Lewis et al., 2007).

1.5.3 Increase anaerobic ATP production

In fish, ATP also can be generated through anaerobic pathways, which primarily include glycolysis yielding lactate accumulation and creatine phosphate (CrP) hydrolysis (Wang & Richards, 2011). These anaerobic pathways are involved in directly transferring phosphate from phosphorylated intermediates, such as glycolytic intermediates or CrP, to ADP producing ATP (Wang & Richards, 2011). Glycogen serves as the carbohydrate store for glycolysis. However, anaerobic metabolism only yields around 3.3% to 6.7% ATP production per mole of substrate consumed compared with ATP generated by oxidative phosphorylation in mitochondria (Richards et al., 2007; Wang & Richards, 2011). Therefore, the ability to survive during severe hypoxia and anoxia will rely on anaerobic capacity, which is determined by the amount of anaerobic fuel available, the usage rate on anaerobic ATP, anaerobic enzyme activities and tolerance to anaerobic toxic-products (lactate and protons) (Nilsson & Östlund-Nilsson, 2008). Previous studies have shown that hypoxia-tolerant fish such as goldfish and crucian carp have higher glycogen levels in their tissues compared with hypoxia-sensitive fish, such as rainbow trout (Wang & Richards, 2011). During anoxia, crucian carp is able to produce ethanol, instead of lactate and protons, as the main anaerobic end-product to escape by diffusion and thus avoiding self-poisoning (Wang & Richards, 2011).

1.6 Genetic variation in hypoxia tolerance

Although many population-based studies have revealed that there is adaption in hypoxia tolerance for species with a broad range of living environments, little is known about the genetic basis of inter-individual variation in hypoxia tolerance in aquatic species. Timmerman & Chapman (2004) proposed that selection pressure for hypoxia tolerance may result in variation among sub-population for species that live in a wide range of oxygen habitats. For example, compared with cyprinid (*Barbus neumayeri*) from well-oxygenated waters, a cyprinid living in a hypoxic swamp had different respiratory traits, such as gill ventilation rate, aquatic surface respiration (Olowo & Chapman, 1996), and total gill filament length (Chapman et al., 1999).

Hypoxia tolerance has been shown to be a repeatable trait in fish (Virani & Rees, 2000; Claireaux et al., 2013; Joyce et al., 2016), suggesting that it may have genetic basis. For example, it has been previously reported that there is a significant heritability of hypoxia tolerance in common carp (*Cyprinus carpio*) (Nagy et al., 1980). A recent study has shown that genetic variation in hypoxia tolerance may exist in the St. John's aquaculture strain of Atlantic salmon due to the high similarity of hypoxia tolerance among full-siblings and half-siblings (Anttila et al., 2013). Claireaux et al. (2013) have shown that European sea bass (*Dicentrarchus labrax*) reared in semi-natural tide ponds displayed a marked interspecific variation in hypoxia tolerance, which is closely associated with survivorship. These observations suggest that hypoxia tolerance in fishes may be genetically determined, at least in part.

In *Drosophila melanogaster*, a whole-genome association study has revealed that there were significant differences in allele frequencies between three hypoxia-tolerant populations and normoxic populations for around 3,800 single nucleotide polymorphisms (SNPs) (Jha et al., 2016). Approximately 50% of these SNPs were clustered into 66 unique genomic regions, which

wwinclude the genes that were expressed differently between hypoxia-tolerant populations and normoxic populations; some of these genes were identified as being associated with metabolic processes and respiratory system development (Jha et al., 2016). Moreover, Jha et al. (2016) have performed a genome-wide association study in the four high-altitude human populations, including Tibetans, Sherpas, Ethiopians and Andeans. Interestingly, they found that many orthologs between hypoxia-tolerant *Drosophila melanogaster* and the four high-altitude human populations were positively selected (Jha et al., 2016). Therefore, hypoxia-tolerance in organisms may be selected based on genetic variation in similar genes and pathways, even in very distantly related organisms.

1.7 Thesis objectives and hypothesis

The major goals of my thesis were to use the strains of farmed-raised Atlantic salmon used by one of British Columbia farmed salmon producers (Marine Harvest) to: i) characterize the variation in hypoxia tolerance, (ii) quantify the genetically-based variation in hypoxia tolerance, (iii) use genotyping by sequencing (GBS) to identify which among the thousands of single nucleotides polymorphisms (SNPs) could be associated with the variation in hypoxia tolerance, and (iv) determine if the significant SNPs that are associated with hypoxia tolerance are shared by smolts in freshwater and adults in seawater. Through the experiments performed in this thesis I have tested the hypothesis that there is a significant association between genetic variation (SNPs) and hypoxia tolerance in Atlantic salmon.

Chapter 2. Genome-Wide Association Study (GWAS) for Hypoxia Tolerance in Atlantic Salmon (*Salmo Salar*) Adults in Sea Cages

2.1 Introduction

The majority of salmon production occurs in sea cages where dissolved oxygen (DO) levels fluctuate substantially with depth, time of day and season (Burt et al., 2012; Johansson et al., 2006; Johansson, et al., 2007; Oppedal et al., 2011). Several studies have demonstrated that hypoxia occurs regularly in sea cages (Johansson et al., 2007; Vigen, 2008), especially in late summer and fall with high water temperature and high stocking density (Johansson et al., 2006; Oppedal et al., 2011), and these hypoxic events have been associated with large-scale mortality events in sea cages (e.g. Blucher, 2015) as well as negative effects on fish growth and health (Buentello et al., 2000; Welker et al., 2007). In addition, anthropogenic climate change is causing higher water temperatures during these times of the year (Hoegh-Guldberg, 1999) and eutrophication is resulting in increased prevalence and severity of hypoxia around sea cages (Braaten, 2007). Thus, hypoxia is already having negative impacts on salmon aquaculture, and it is likely that these impacts will only become more severe in the future unless measures are employed to mitigate the effects of hypoxia when salmon are held in sea cages.

During periods of hypoxia, fish face the challenge of a reduced aerobic ATP production through oxidative phosphorylation in mitochondria. The reduced aerobic ATP production due to hypoxia has negative effects on fish survival (Azanza, et al., 2005), growth rate and feed conversion efficiency (Buentello, et al., 2000; Oppedal et al., 2011), increased susceptibility to diseases (Welker et al., 2007), and reproduction (Wu, 2009). Fish generally respond to hypoxia

to mitigate these effects through a series of behavioural, physiological, biochemical and molecular adjustments that enhance oxygen extraction from the hypoxic environments to the tissues or reduce the consequences of oxygen shortage in the tissues (Richards, 2011). For example, previous studies have shown that fish exposed to hypoxia increase gill surface area (Nilsson, 2007), increase gill ventilation and perfusion (Perry, 2011; Perry et al., 2009), and increase Hb-O_2 binding affinity (Wells, 2009) to enhance oxygen extraction. At DO levels low enough to impair oxygen extraction even after compensatory responses such as those described above, the fundamental challenge for fish is metabolic energy balance (Farrell & Richards, 2009). Because these conditions result in reduced oxygen supply and limitation of aerobic metabolism, fish must depend on anaerobic metabolism to support metabolic demand (Wang & Richards, 2011) and suppress metabolic demand to maintain energy balance for hypoxia survival (Hochachka, et al., 1996). Despite our general understanding of how hypoxia impacts fish and the mechanisms through which fish respond to hypoxia, few studies have attempted to elucidate the genetic basis of variation in hypoxia tolerance in fish.

Over the past decade, the fast growth in genotyping technologies has significantly enhanced the tools available to explore the genetic basis of variation in many complex traits (Gutierrez et al., 2012; Korte & Farlow, 2013). In particular, genotyping by sequencing (GBS) approaches have provided a powerful platform to identify large numbers of single nucleotide polymorphisms (SNPs) across the genome that can be used for constructing the genotype-phenotype map, in what is known as a Genome-wide Association Study (GWAS) (Elshire et al., 2011). GWAS has been a prevailing approach to identify genetic variants associated with phenotypic traits of interest in humans, animals and plants (Begum et al., 2015; Korte & Farlow, 2013). Numerous convincingly associated loci have been detected through GWAS, for example,

40 loci for human height (Gudbjartsson et al., 2008; Lettre et al., 2008; Weedon et al., 2008), 15 loci for type 2 diabetes (Mohlke et al., 2008) and 4 loci for harvest weight in Atlantic salmon (Tosh et al., 2014).

The power of GWAS to detect a true causal variant that leads to phenotypic variation is dependent on the levels of linkage disequilibrium (LD) between the genetic markers and the true causal variants (Gutierrez et al., 2015; Sodeland et al., 2013), where LD is a measure of the degree of non-random allelic association between different loci (Slatkin, 2008). Levels of LD in a population are affected by evolutionary process such as natural selection, genetic drift, recombination rate and mutation rate (Slatkin, 2008). Farmed Atlantic salmon have been previously reported to have long-range LD, and this may be due to a recent admixture in a 'synthetic' breeding population that originally came from several distinct rivers (Moen et al., 2008). As a result, this long-ranging LD in farmed Atlantic salmon is advantageous for GWAS and also increases the power to identify the true causal variants that result in phenotypic variation (Sodeland et al., 2013).

In the present study, we performed a GWAS to identify the genetic bases of variation in hypoxia tolerance in the McConnell and Mowi strains of Atlantic salmon, which are commonly reared in British Columbia (BC) by Marine Harvest Canada (an aquaculture company based primarily on Vancouver Island). The objectives of this study were to: i) identify if there is phenotypic variation in hypoxia tolerance in adult Atlantic salmon; (ii) use a genotyping by sequencing (GBS) approach to identify single nucleotides polymorphisms (SNPs) in these fish, and iii) perform a GWAS to identify which among these thousands of (SNPs) in adult Atlantic salmon could be associated with the variation in hypoxia tolerance.

2.2 Methods

2.2.1 Fish husbandry

Atlantic salmon were reared from egg to smolt stage at the Marine Harvest Freshwater Farms Hatchery in Duncan, British Columbia, Canada. During the spawning season (November/December 2013), a total of 291 mating families were established including fish of the Scottish McConnell strain and the Norwegian Mowi strain, as well as hybrid crosses between the two strains. These two European strains of Atlantic salmon were imported to BC in the 1980s and 1990s, respectively, (Withler, Supernault, & Miller, 2005) and maintained in the BC industry since then. After fertilization, 19 eggs from each family were randomly selected and kept in a heath tray, yielding a total number of 5,529. At the fry stage (April 2014), all the fish from the heath tray were moved into a 9,300-l tank. The fish were reared in flow-through well-water in 24 h light, followed by ambient light starting on June 21, 2014. Water temperature ranged from 10 to 16 °C year around. Oxygen saturation was maintained between 80% and 120% (a Point Four System monitor dissolved oxygen concentration continuously). An automatic feeder was used to feed fish with a daily feed ration of 1 to 1.2% of body mass. After reaching the smolt stage, the fish were moved into a 35,000-l tank. The smolt stage was identified using real-time PCR to detect the expression of NKAα1b (Na^+/K^+-ATPase α-1b isoform), which is the major isoform expressed during smolt development and used for seawater adaption (McCormick et al., 2013). At around 200 g (April 2015), approximately 4,500 of the Atlantic salmon smolts were implanted with PIT (passive integrated transponder) tags and fin clips were collected. DNA was extracted from the fin clips of PIT-tagged smolts and from the parents that were used to produce the 291 families. The DNA then was genotyped at eight microsatellite loci for parental assignment by Ruth Withler and her colleagues at Fisheries and Oceans Canada (DFO) in

Nanaimo (Withler et al., 2007). Within the same month (April 2015), the tagged fish were confirmed to be in the smoltification window and transferred to a smolt sea pen at Potts Bay, which is a Marine Harvest seawater broodstock site. The fish in seawater were fed to satiation daily. Daily water quality monitoring including salinity, DO, temperature, plankton and turbidity. In February 2016 when the fish reached approximately 2,200 g, a subset of the fish were haphazardly selected to test for hypoxia tolerance.

2.2.2 Phenotyping: hypoxia tolerance

To test hypoxia tolerance of adults in seawater, batches of approximately 20 fish were haphazardly selected from the sea pen and transferred to the experimental tank (around 1,100 L). Bubble wrap was used to seal the surface of water so that oxygen in the air could not diffuse to the water. Fish were allowed to adjust to the new environment for 30 min during which dissolved oxygen in the experimental tank was maintained above 80% by bubbling oxygen into the experimental tank. A submersible pump was used to mix the water in the experimental tank and an oxygen meter (OxyGuard Handy Polaris) was used to monitor oxygen saturation in the water. After this initial period, nitrogen was introduced into the water to decrease dissolved oxygen at a rate of 2% per min until oxygen reached 22% of air saturation (2.1 mg/L DO). A timer started recording once dissolved oxygen reached 22% of air saturation and the time at which individual fish lost equilibrium (LOE) was recorded. Fish that lost equilibrium were immediately removed from the tank and stunned by percussion before body mass, fork length and sex were measured. PIT tag numbers were recorded, and fin clips were collected and stored in 95% ethanol for extracting DNA. A total of 198 Atlantic salmon adults were tested for hypoxia tolerance among 8 trials.

2.2.3 Genotyping

2.2.3.1 DNA preparation

DNA was isolated from the fin clip samples using a Qiagen DNeasy Blood & Tissue Kit (Cat No. 69506). A tissue sample of about 20 mg was cut into small pieces and incubated in lysis buffer with proteinase K at 56 °C overnight to allow complete digestion of the sample. The samples were then treated with RNase Cocktail (Thermo Fisher Scientific) and DNA was isolated according to the manufacturer's instructions. In total, DNA was extracted from 190 adults, randomly selected from the animals that were phenotyped for hypoxia tolerance, as described above.

Overnight digestions of and aliquot of 10% of the samples were performed with the restriction enzyme HindIII at 37 °C. Undigested and digested DNA samples were run on 1% agarose gels using SYBR Safe DNA Gel Stain (Invitrogen). These gel images were used to determine the quantity and quality of the DNA samples (i.e. to ensure DNA was high molecular weight and capable of effective digestion). Undigested DNA samples were quantified using Quant-IT^{TM} PicoGreen$^{@}$ dsDNA Assay Kit (Invitrogen) with a fluorometer. Based on readings from the fluorometer, all DNA samples were diluted to 100 ng/µL before submission to the Genomic Diversity Facility at Cornell University.

2.2.3.2 Library preparation

Genotyping-by-sequencing was completed at Institute of Biotechnology, Cornell University (using a protocol based on Elshire et al., 2011). The restriction enzyme *Eco*T22I was used to digest genomic DNA to decrease genome complexity. Two types of adaptors were then used to ligate to the ends of digested DNA products; one adaptor was a barcode adaptor to

identify each sample; the other was an adaptor with an *Eco*T22I compatible sticky end. After the ligation, all the ligated products were pooled into a single tube and then purified. A PCR was then performed to amplify the ligated products with the primers that were complementary to the sequences of adaptors and 3' sequences compatible with the sequences on the surface of the Illumina sequencing flow-cell were added by the PCR primers. After PCR, all the fragments were cleaned and purified using a commercial kit (QIAquick PCR Purification Kit; Qiagen), and the size of all fragments were evaluated on a DNA analyzer (BioRad Experion). The pooled fragments were quantified and loaded for sequencing on an Illumina Hiseq 2500.

2.2.3.3 SNP discovery by GBS

Raw sequencing data from Illumina Hiseq 2500 were analyzed using the GBS pipeline in Tassel 3.0 standalone as described by Elshire et al. (2011) and Glaubitz et al. (2014). This approach first filtered for raw sequencing reads based on the three parameters, which included those perfectly matched one of the barcodes, those with no "Ns" in their first 72 bases, and those with no adapter dimers. The subsequent filtering of reads was done in two ways. First, the sequencing data were filtered for unique sequence "tags", which were used to map to a reference genome. Only "tags" that were present at least 3 reads were remained for downstream analysis. The "tags" were then aligned to the Atlantic salmon genome (assembly ICSASG_v2) for SNP calling. Also, all the sequencing reads were sorted into different files based on their barcodes; the barcode in each read was then removed, and all the reads were trimmed to 64 bases. As a result, each individual had a unique sequencing file and the genotype of individuals was called based on this information.

Further filtering was used to remove SNPs that had call rates < 80% and minor allele frequencies (MAF) < 0.02. SNPs were kept only if they were bi-allelic loci, and individuals with over 60% missing data were removed from the filtered genotype dataset. This filtering removed 5 individuals from the dataset. Following a whole-genome duplication event experienced by the common salmonid ancestor, the paralog retention rate in salmonids remained very high, ranging from 25% to 75% (Bailey et al., 1978). A recent study has also reported that Atlantic salmon exhibited an over 90% similarity in a large proportion of homeologous blocks (573 Mb) (Lien et al., 2016). These duplicated blocks can potentially lead to mapping errors in sequence alignment during GBS, resulting in false SNP calling. To solve the problem, McKinney et al. (2016) developed the *HDplot* approach to identify paralogous loci based on the expected heterozygotes within a population (*H*) and the expected allelic ratios from the heterozygous individuals (*D*). According to the findings for Chinook salmon (*Oncorhynchus tshawytscha*), a close relative of Atlantic salmon, the same *HDplot* values were used for this analysis: $-7 \leq D \leq 7$ and $H \leq 0.6$ (McKinney et al., 2016). As a result, the dataset retained 27,362 SNPs, which were used for association analyses.

2.2.4 Population structure analysis

A challenge with GWAS is to deal with false-positive associations due to population structure (Bradbury et al., 2007; Price et al., 2010). The majority of false-positive associations that previously have been reported occur at markers with large allele frequency differences between subpopulations (Campbell et al., 2005; Tian et al., 2008). Nevertheless, principal components analysis (PCA) has been successfully used to reduce this risk arising from population structure (Zhao et al., 2007). Thus, PCA was performed in this study to evaluate the

population structure using Tassel 5.0 software (Bradbury et al., 2007). The final total of 27,362 SNPs in the dataset was used for PCA analysis.

2.2.5 Kinship analysis

Family structure is another factor that can cause false-positive results in GWAS (Price et al., 2010). However, kinship analysis has been reported to effectively reduce false positives arising from family structure (Price et al., 2010). In this study, kinship analysis was performed to evaluate the family structure using Tassel 5.0 software (Bradbury et al., 2007). The final total of 27,362 SNPs in the dataset was used for kinship analysis.

2.2.6 Genome-wide association study (GWAS)

To identify a SNP-Trait association in hypoxia tolerance in Atlantic salmon, a mixed linear model (MLM) in Tassel version 5.0 was used to carry out genome-wide association studies. In addition, the mixed linear model was also used to perform a SNP-Trait association in body mass in Atlantic salmon. The MLM included population structure (PCA) and family relatedness (Kinship) and can be defined by the following formula, Henderson's matrix notation (Henderson, 1975):

$$y = X\beta + Z\mu + e$$

where y is the vector of phenotypic data (time-to-loss-of-equilibrium at 22% of air saturation); β is the vector of fixed effects, which include SNPs and population structure (PCA); μ is the vector containing random additive genetic effects; X and Z are the designed matrices for β and u respectively; e is the vector containing random residuals. For this model, the u and e vectors were assumed to be normally distributed: $u \sim N(0, K\delta^2_a)$ and $e \sim N(0, I\delta^2_e)$; K is kinship

matrix and δ^2_a is the polygenic additive variance; I is an identified matrix and δ^2_e is the residual variance. False discovery rate (FDR) method was used to assess the significance of SNPs (Benjamini & Hochberg, 1995).

2.2.7 Candidate gene analysis

To identify candidate genes for hypoxia tolerance, significant SNPs were searched for candidates by using the Integrative Genomics Viewer (IGV) on Atlantic salmon genome (ICSASG_V2). A gene was considered a candidate gene if it contained a significant SNP or the gene contained a SNP that is linkage disequilibrium with a significant SNP.

2.2.8 Statistical analysis

The data of hypoxia tolerance at family level was analyzed using a Kruskal-Wallis test in R. The data of hypoxia tolerance at strain level was analyzed using Kruskal-Wallis one way ANOVA and pairwise comparison tests in R.

2.3 Results

2.3.1 Hypoxia tolerance

2.3.1.1 Variation in hypoxia tolerance among individuals of Atlantic salmon

A large variation in hypoxia tolerance was observed among individual adult Atlantic salmon, with time-to-LOE at 2.1 mg/L DO ranging from 4.6 min to 126.9 min (Fig. 2.1). The majority of adults were hypoxia-sensitive and hypoxia-tolerant individuals were rare. Specifically, approximately 85% of adults lost equilibrium within 30 min and only 7% of adults tolerated over 60 min.

2.3.1.2 Hypoxia tolerance at family level

Adults were separated into 91 families based on the results of microsatellite genotyping, and only families with at least 3 individuals were used for detection of variation in hypoxia tolerance at the family level. Thirty families met the requirement of which 3 families were the McConnell fish, 24 families were the Mowi fish and 3 families were hybrid fish (Fig. 2.2). A wide range of variation in time-to-LOE was observed among families, with mean values ranging from 9.2 min to 69.4 min (Fig. 2.2). However, there was no significant difference in time-to-LOE at family level (Kruskal-Wallis test, $P = 0.094$), but the low number of individuals within each family (23 out of 30 families had only 3 individuals per family) may have resulted in low statistical power for this analysis.

2.3.1.3 Hypoxia tolerance at strain level

Adults were separated into Mowi strain, McConnell strain and hybrids, according to the results from microsatellite genotyping. The total of 198 adults contained 27 individuals from the McConnell strain, 153 from the Mowi strain, and 18 from the hybrid. There was a significant difference in hypoxia tolerance among these groupings, with the median of time-to-LOE exhibiting 22.3 min in McConnell, 18.7 min in Mowi, and 14.4 min in hybrids (Kruskal-Wallis one way ANOVA, $P = 0.0015$). The post-hoc tests demonstrated that the McConnell strain had a significantly longer time-to-LOE than the Mowi strain ($P = 0.0022$), whereas LOE of neither the McConnell strain nor the Mowi strain differed significantly from the LOE of the hybrids ($P = 0.65$ and $P = 0.19$, respectively) (Fig. 2.3). Large variation in hypoxia tolerance was also observed within these strains of Atlantic salmon. Specifically, time-to-LOE among McConnell

individuals ranged from 4.2 min to 79 min, whereas time-to-LOE for Mowi individuals ranged from 0.8 min to 126.9 min (Fig. 2.3).

2.3.2 Body mass

Body mass varied substantially among adult Atlantic salmon, ranging from 213 to 4,260 g, with a median of 2,145 g (Fig. 2.4). At the family level, a significant difference in body mass was identified (Kruskal-Wallis test, $P = 0.0063$). However, there was no significant difference in body mass among the strains of Atlantic salmon adults (Kruskal-Wallis test, $P = 0.488$).

2.3.3 Genotyping by sequencing

A total of 185 samples and 27,362 SNPs in the dataset passed the quality control and were used for association analyses. The number of SNPs per chromosome ranged from 265 on chromosome 8 (NC_027307.1) to 2,037 on chromosome 1 (NC_027300.1) (Fig 2.5). The average of 12 SNPs per megabase (Mb) was identified for the entire genome, and the proportion of SNPs by chromosome ranged from 9 to 15 SNPs per megabase (Fig 2.5).

2.3.4 GWAS for Atlantic salmon

A GWAS analysis was performed for hypoxia-tolerance in Atlantic salmon adults in seawater. A total of 8 SNPs was identified as significantly associated with hypoxia tolerance in Atlantic salmon adults according to the significance threshold as determined by a false discovery rate of 0.05 (Fig 2.6). The top two SNPs (S1_982279883 and S1_982279912) were significantly associated with hypoxia tolerance at the genome-wide level of significance ($P = 7.69e-07$ and $P = 7.69e-07$). The other six SNPs (S1_871678299, S1_871818843, S1_871872804,

S1_1471888992, S1_867269825 and S1_871007038) were significantly associated with hypoxia tolerance at the chromosome-wide level of significance (*P* = 8.78e-05, *P* = 8.56e-05, *P* = 8.96e-05, *P* = 4.86e-05, *P* = 1.52e-04 and *P* = 1.56e-04 respectively). Importantly, SNP S1_982279883 and SNP S1_982279912 were only 29 bp apart and located nearby mitochondrial fission process protein 1-like (Mtfp1) on chromosome 11 (NC_027310.1). The next three most significant SNPs (S1_871678299, S1_871818843 and S1_871872804) that were identified were on chromosome 10 (NC_027309.1). These three SNPs were located in calcium-dependent secretion activator 2 (CADPS2), nearby protein tyrosine phosphatase, receptor type Z1 (PTPRZ1), and in PTPRZ1 respectively. SNP S1_1471888992 was located nearby activin receptor type-2A (ACVR2A) on chromosome 16 (NC_027315.1). The last two significant SNPs, S1_867269825 and S1_871007038, were located in cation channel sperm associated 2 and metabotropic glutamate receptor 8-like respectively on chromosome 10 (NC_027309.1) (Table 2.1).

Additionally, we performed a GWAS for body mass in adult Atlantic salmon. Four SNPs were identified at chromosome-wide level of significance (Fig 2.6). The most significant SNP (S1_1570467139) was found in uncharacterized LOC 106576981 on chromosome 18 (NC_027317.1). The next three significant SNPs (S1_224141594, S1_179657087 and S1_204440772) were all located on chromosome 2 (NC_027301.1). SNP S1_224141594 and SNP S1_179657087 were located in lysosomal alpha-glucosidase-like and uncharacterized LOC106579645 respectively. SNP S1_204440772 was located around 19 kb upstream of regulatory-associated protein of mTOR (RAPTOR).

2.4 Discussion

This study is the first to carry out GWAS to identify genetic variants associated with hypoxia tolerance in Atlantic salmon using high-density SNP markers. Our goals were to characterize the variation in hypoxia tolerance and identify the genetic basis of variation in hypoxia tolerance in adult Atlantic salmon. The four major findings were that: i) substantial variation in hypoxia tolerance exists among individuals of Atlantic salmon; (ii) a strain effect on hypoxia tolerance exists, with McConnell strain having better hypoxia tolerance than Mowi strain; (iii) two SNPs were identified at the genome-wide level of significance (S1_982279883 and S1_982279912) and six SNPs at the chromosome-wide level of significance (S1_871678299, S1_871818843, S1_871872804, S1_1471888992, S1_867269825 and S1_871007038) associated with hypoxia tolerance in Atlantic salmon; and (iv) we identified four SNPs (S1_1570467139, S1_224141594, S1_179657087 and S1_204440772) associated with body mass at the chromosome-wide level of significance.

2.4.1 Hypoxia tolerance

Salmonid species are generally considered to be very sensitive to hypoxia (Doudoroff & Shumway, 1970; Dunn & Hochachka, 1986) and while our results are consistent with this general idea, some individual Atlantic salmon are much more tolerant of hypoxia than would be expected based on the average tolerance of the species. For instance, 13 adults in seawater were able to tolerate 2.1 mg/L DO for more than 60 min compared to a median LOE of 16 min. A previous study on the Tasmanian stock of Atlantic salmon has also shown that some individuals were able to tolerate a relatively high degree of hypoxia (Barnes et al., 2011). Considerable individual variability of hypoxia tolerance is well established for the European sea bass

(*Dicentrarchus labrax*) too, and this variability is directly associated with survivorship in semi-natural tidal ponds (Claireaux et al., 2013; Joyce et al., 2016).

The wide range of variation in individual hypoxia tolerance in adult Atlantic salmon is very important finding because phenotypic variation is one component that is required for artificial or natural selection to be successful (e.g. López et al., 2014). Similar results were found by Anttila et al. (2013), who showed large variation in hypoxia tolerance in the St. John strain of Atlantic salmon, with the range for time-to-LOE being similar (114.8 min) to that obtained here (126.1 min). This consistency is apparent, even though the previous hypoxia challenge was performed at 4 °C versus 10.5 °C used in this study. Elevated water temperatures can lead to decreased hypoxia tolerance (Vaquer-Sunyer & Duarte, 2011), while thermal acclimation has potential to partially mitigate these effects (McBryan et al., 2016).

While we also observed substantial inter-family variation in hypoxia tolerance in adult Atlantic salmon, with the mean time-to-LOE ranging from 9.2 min to 69.4 min, there was no statistically significant difference in hypoxia tolerance among families of Atlantic salmon, perhaps because of a low statistical power to detect a difference due to the low number (3) of individuals per family. Previous studies in several species of fish have revealed significant difference in hypoxia tolerance at the family level. For example, Anttila et al. (2013) demonstrated that the St. John strain of Atlantic salmon had higher similarity of hypoxia tolerance among full-siblings than half-siblings. Likewise, Nagy et al. (1980) showed that there was a significant heritability of hypoxia tolerance in common carp (*Cyprinus carpio*).

Previously, no studies have investigated inter-strain variation in hypoxia tolerance in Atlantic salmon. Nevertheless, a clear strain effect on hypoxia tolerance was revealed in the present study, with the McConnell strain being more hypoxia tolerant than the Mowi strain. This

result is consistent with the inter-strain variation in hypoxia tolerance observed in other salmonid species. For instance, differences in hypoxia tolerance have previously been observed among strains in juvenile rainbow trout (Scott et al., 2014).

2.4.2 GWAS for hypoxia tolerance

Our GWAS was able to identify two SNPs (SNP S1_982279883 and SNP S1_982279912) significantly associated with hypoxia tolerance in Atlantic salmon at the genome-wide level of significance. These SNPs are only 29 bp apart and located nearby mitochondrial fission process protein 1 (Mtfp1) (around 8 kb upstream of the SNPs). Mtfp1, also known as MTP18, is a mitochondrial protein that impacts mitochondrial morphology and causes apoptosis. Moreover, Mtfp 1 is a transcriptional downstream target of the phosphatidylinositol (PI) 3-kinase signaling pathway (Tondera et al., 2004). PI 3-kinase has been widely studied and plays a role in regulating cell development, growth, adhesion, glucose transport, motility, immune response and survival (Katso et al., 2001). Baregamian et al. (2007) has shown that hypoxia-inducible factor (HIF)-1 is regulated by PI 3-kinase pathway to protect hypoxic injury in neonatal mice during necrotizing enterocolitis. Hypoxia-inducible factor (HIF)-1is a dimeric protein complex that plays critical role in the response to hypoxia in mammals (Shen et al., 2005; Ziello et al., 2007).

At the chromosome level of significance, six SNPs (S1_871678299, S1_871818843, S1_871872804, S1_1471888992, S1_867269825 and S1_871007038) were identified as significantly associated with hypoxia tolerance in Atlantic salmon adults. SNP S1_871678299 was located within calcium-dependent secretion activator 2 (CADPS2). CADPS2 is a calcium binding protein that regulates the exocytosis of synaptic and dense-core vesicles in neurons and

neuroendocrine cells (Ashkov, 1979). SNP S1_871818843 was found close to a gene coding for protein tyrosine phosphatase, receptor type Z1 (299 bp away from the SNP), and SNP S1_871872804 was identified in protein tyrosine phosphatase, receptor type Z1 (PTPRZ1). PTPRZ1 is suggested to play a role in the regulation of specific developmental process in the central nervous system (Kaper, 1976). SNP S1_1471888992 is closest to a gene coding for activin receptor type-2A (ACVR2). ACVR2 belongs to the transforming growth factor beta (TGF-β) receptor family(Heller et al., 1975). SNP S1_867269825 was identified in cation channel sperm associated 2 (CATSPER2), which belongs to a member of cation channel protein gene family that mediates Ca^{2+} influx in sperm and is essential for male fertility (Ren et al., 2001). The final identified SNP, S1_871007038, is located within a gene coding for metabotropic glutamate receptor 8-like (mGluR8). mGluR8 belongs to the G-protein-coupled receptor (GPCR) superfamily that plays an important role in adjusting synaptic transmission and neuronal excitability throughout the central nervous system (Niswender & Conn, 2010). Interestingly, a previous study showed that the group II metabotropic glutamate receptors were involved in defending against brain damage in goldfish during anoxic exposure (Poli et al., 2003).

2.4.3 GWAS for body mass

Four SNPs (S1_1570467139, S1_224141594, S1_179657087, S1_204440772) were identified to be significantly associated with body mass, but only at the chromosome level of significance. SNP S1_1570467139 was found in uncharacterized LOC 106576981 on chromosome 18 (NC_027317.1). SNP S1_224141594 was found in lysosomal alpha-glucosidase-like, and its function is to degrade glycogen to glucose in lysosomes (Hermans et al.,

1993). SNP S1_179657087 was located in uncharacterized LOC106579645. Interestingly, SNP S1_204440772 was found approximately 19 kb upstream of regulatory-associated protein of mTOR (RAPTOR). This gene is involved in controlling the mammalian target of rapamycin complex 1 (mTORC1) that regulates cell growth, metabolism and survival (Hori et al., 2013). Furthermore, Bond (2016) has demonstrated that mTORC1 plays an important role in regulating skeletal muscle protein synthesis.

2.5 Conclusion

The main purpose of this study was to identify the genetic markers that are significantly associated with hypoxia tolerance in the farmed Atlantic salmon used by Marine Harvest Canada and that can be incorporated into a breeding program using maker assisted selection. We identified two SNPs using genome-wide FDR correction and six SNPs using chromosome-wide FDR correction, and these SNPs could be potentially used by Marine Harvest Canada for marker-assisted selection. In this study, we also found that strain had an effect on hypoxia tolerance in Atlantic salmon, with McConnell strain having better hypoxia tolerance than Mowi strain. In addition, we identified four significant SNPs associated with body mass with chromosome-wide FDR correction.

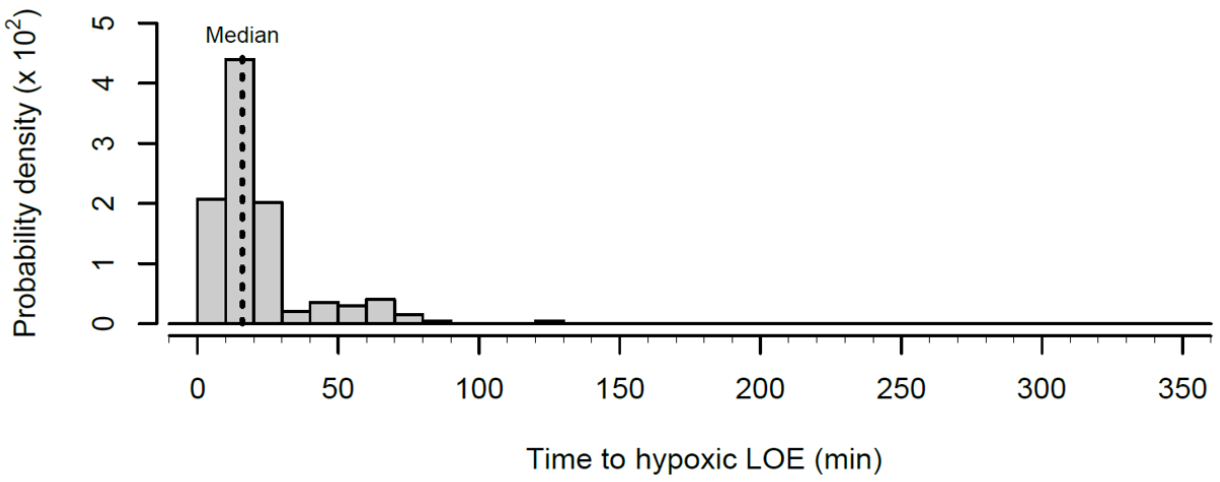

Figure 2.1 The distribution of hypoxia tolerance in Atlantic salmon. Hypoxia tolerance was measured as time-to-loss of equilibrium at 2.1 mg/L oxygen. A total of 198 adults in seawater were tested for hypoxia tolerance. The median of the time-to-LOE was 16 min.

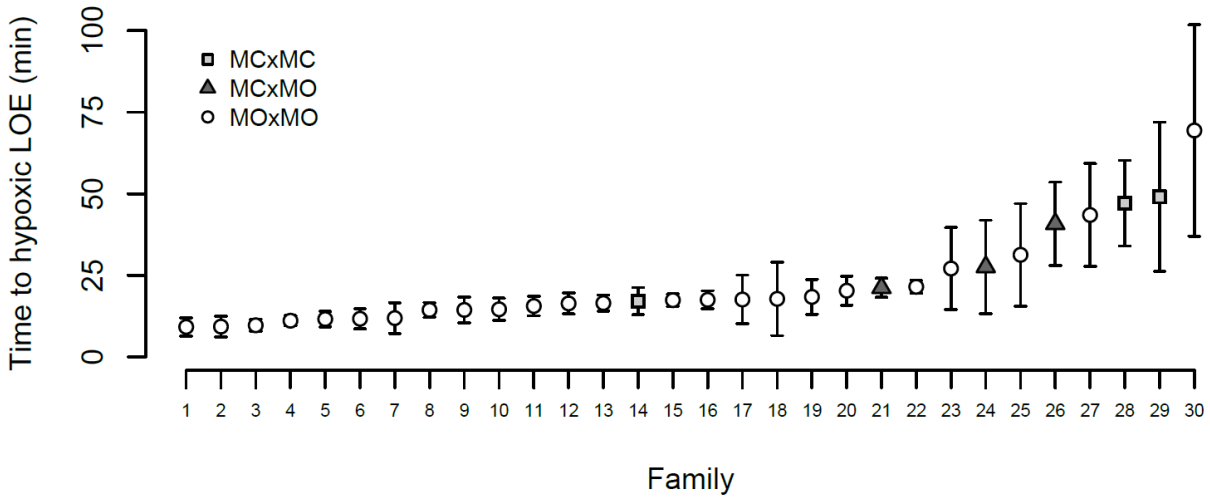

Figure 2.2 The rank order of mean (± SD) time-to-LOE during hypoxia challenge tests for 30 families of Atlantic salmon adults in seawater. Each family contained at least three individuals, and the family mean value ranged from 9.2 min to 69.4 min. The open circle indicates the Mowi strain, the square indicates the McConnell strain, and the triangle indicates hybrids.

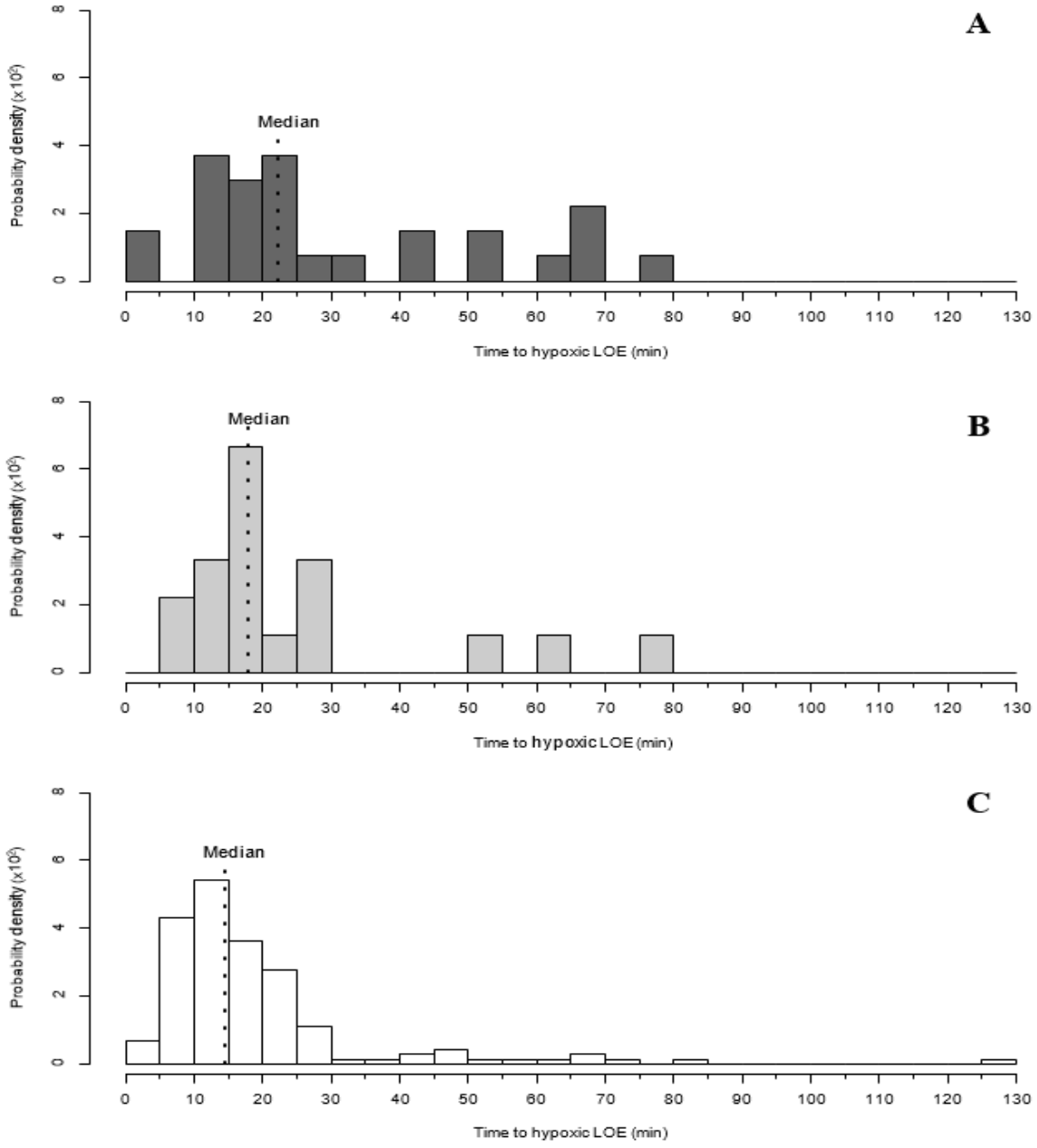

Figure 2.3 The distribution of hypoxia tolerance among three strains of Atlantic salmon McConnell (Panel A), hybrids (Panel B) and Mowi (Panel C) (A) the McConnell strain contains 27 individuals, and the median time-to-LOE was 22.3 min (B) the hybrids contain 18 individuals, and the median time-to-LOE was 18.7 min. (C) the Mowi strain contains 153 individuals, and the median time-to-LOE was 14.4 min. There is a significant difference in hypoxia tolerance among the three strains (Kruskal-Wallis one way ANOVA, $P = 0.0015$). The McConnell strain had a significantly longer time-to-LOE than the Mowi strain ($P = 0.0022$), whereas neither the McConnell strain nor the Mowi strain differed significantly in time-to-LOE from the hybrids (Pairwise comparison tests, $P = 0.65$; $P = 0.19$).

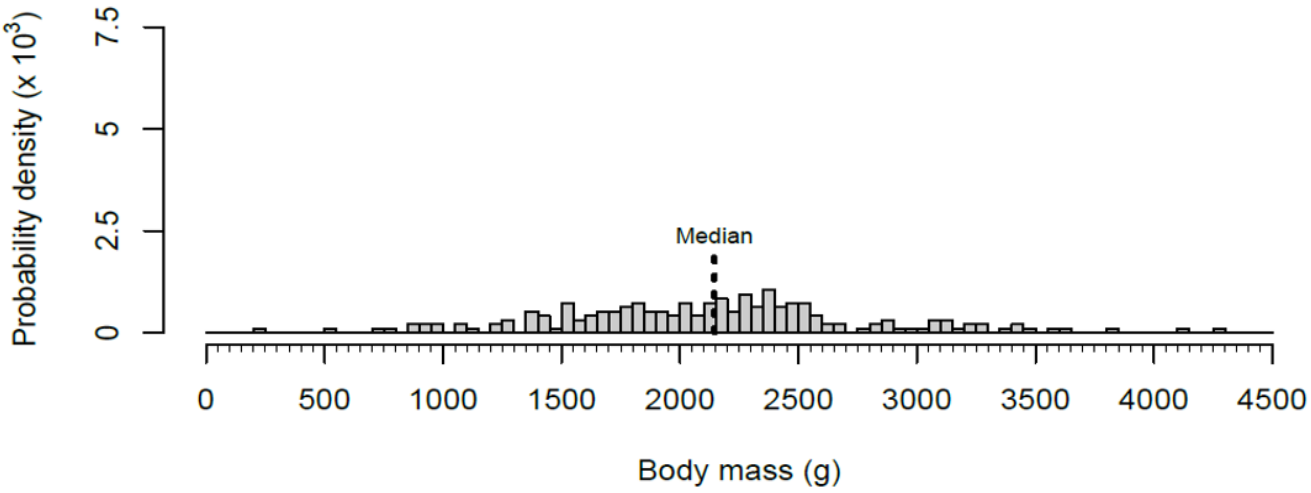

Figure 2.4 The distribution of body mass in Atlantic salmon adults in seawater. A total of 192 adults were measured for body mass and the median was 2,145 g.

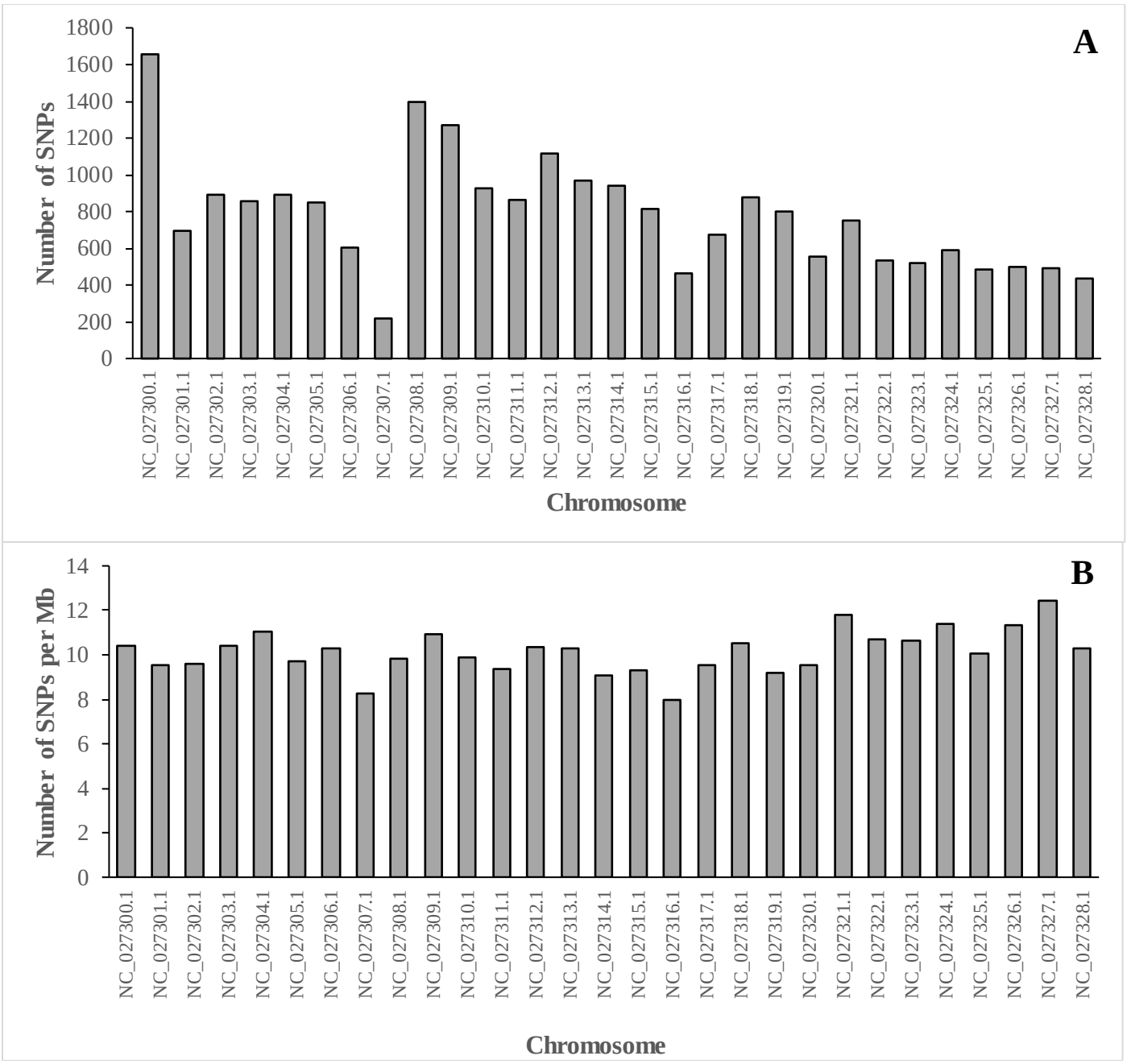

Figure 2.5 (A) The total number of SNPs identified across the Atlantic salmon genome and (B) the proportion of SNPs by chromosome calculated in SNPs/Mb.

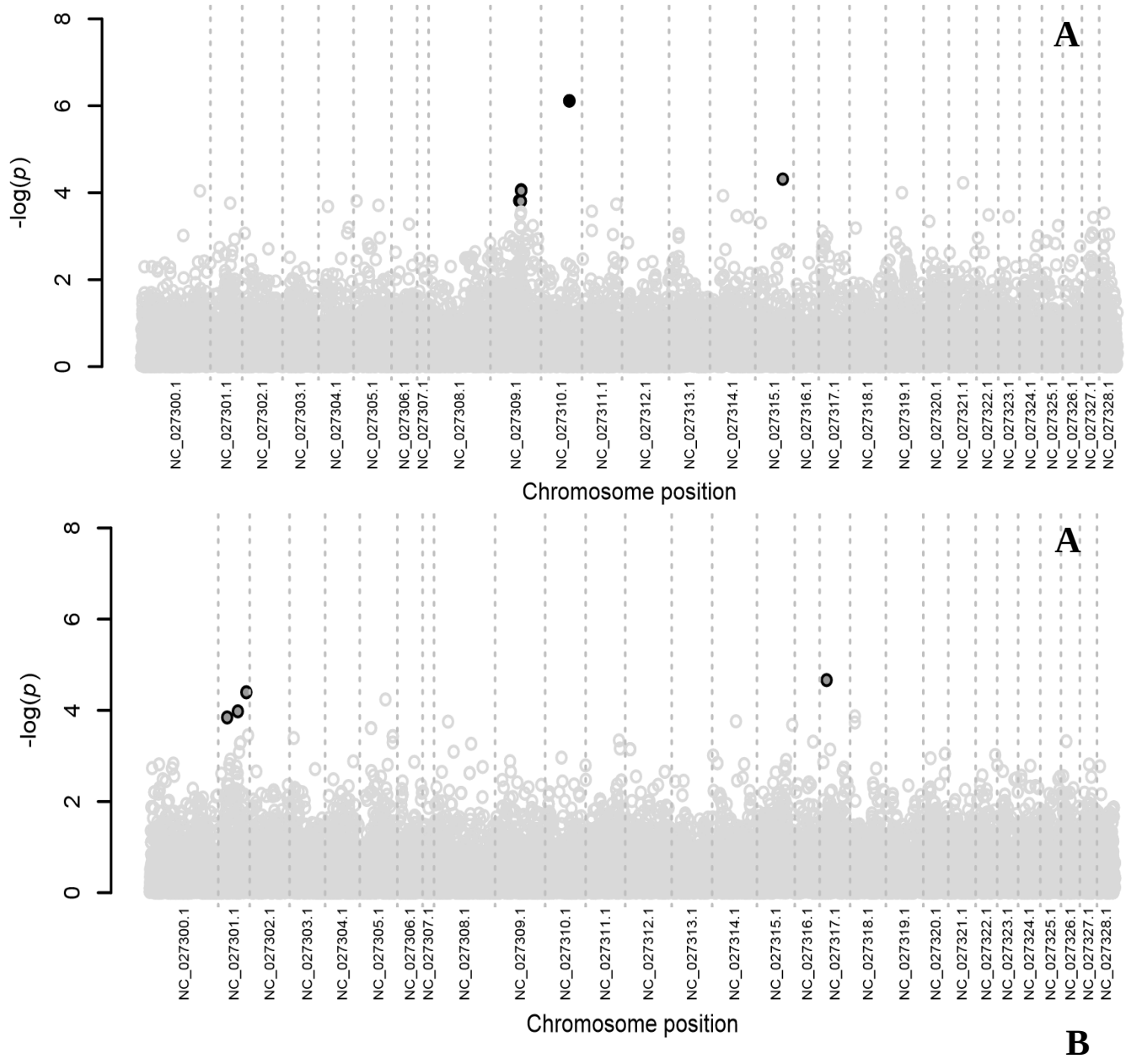

Figure 2.6 Manhattan plots displaying the significance of associations between each identified SNP and phenotypes in Atlantic salmon adults. (A) hypoxia tolerance, (B) body mass. The black dots represent SNPs that are significantly associated with the specified trait with genome-wide FDR correction, and the grey dots indicate SNPs that are significantly associated with the specified trait with chromosome-wide FDR correction.

Table 2.1 Hypoxia tolerance and body mass association identified in the analyses. Chromosome, alleles, genes, *P*-values and marker r^2 of significant SNPs at the genome level of significance and chromosome level of significance for hypoxia tolerance and body mass in Atlantic salmon adults.

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Hypoxia	Genome	S1_982279883	NC_027310.1	-/T	Nearest (around 8 kb) mitochondrial fission process protein 1-like	7.69e-07	0.13
Hypoxia	Genome	S1_982279912	NC_027310.1	-/A	Nearest (around 8 kb) mitochondrial fission process protein 1-like	7.69e-07	0.13
Hypoxia	Chromosome	S1_871678299	NC_027309.1	A/G	Calcium-dependent secretion activator 2	8.78e-05	0.09
Hypoxia	Chromosome	S1_871818843	NC_027309.1	C/T	Nearest (299 bp) protein tyrosine phosphatase, receptor type Z1	8.56e-05	0.09
Hypoxia	Chromosome	S1_871872804	NC_027309.1	C/G	Protein tyrosine phosphatase, receptor type Z1	8.96e-05	0.09
Hypoxia	Chromosome	S1_1471888992	NC_027315.1	A/T	Nearest activin (142 kb) receptor type-2A	4.86e-05	0.12
Hypoxia	Chromosome	S1_867269825	NC_027309.1	A/G	Cation channel sperm associated 2	1.52e-04	0.09
Hypoxia	Chromosome	S1_871007038	NC_027309.1	C/T	Metabotropic glutamate receptor 8-like	1.56e-04	0.10

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Body mass	Chromosome	S1_1570467139	NC_027317.1	-/A	Uncharacterized LOC106576981	2.17e-05	0.11
Body mass	Chromosome	S1_224141594	NC_027301.1	-/A	Lysosomal alpha-glucosidase-like	4.01e-05	0.09
Body mass	Chromosome	S1_179657087	NC_027301.1	-/C	Uncharacterized LOC106579645	1.43e-04	0.08
Body mass	Chromosome	S1_204440772	NC_027301.1	T/G	Nearest (around 19 kb) regulatory-associated protein of mTOR	1.05e-04	0.08

Chapter 3. Genome-Wide Association Study (GWAS) for Hypoxia Tolerance in Atlantic Salmon (*Salmo Salar*) Smolts in Freshwater

3.1 Introduction

Although exposure to environmental hypoxia is more likely to occur when Atlantic salmon are held in the natural environment in sea cages, nevertheless, the early life stage in hatcheries may also experience hypoxia due to some unintentional factors (e.g. pump failure) and during transport from freshwater hatcheries to sea cage sites. Therefore, in this chapter, I examined levels of variation in hypoxia tolerance among smolts of the same brood year of the adult fish used in Chapter 2, and performed a genome-wide association study (GWAS) to attempt to identify genetic variation that is associated with variation in hypoxia tolerance. If common markers associated with hypoxia tolerance exist between smolts and adults, these would represent particularly good candidates for marker assisted selection.

The objectives of this study were to: i) identify if there is phenotypic variation in hypoxia tolerance in Atlantic salmon smolts; (ii) use a genotyping by sequencing (GBS) approach to identify single nucleotides polymorphisms in (SNPs), (iii) perform a GWAS to identify which among the thousands of single nucleotides polymorphisms in Atlantic salmon (SNPs) could be associated with the variation in hypoxia tolerance; and (iv) determine whether there are common SNPs associated with hypoxia tolerance between smolts in freshwater and adults in seawater.

3.2 Methods

3.2.1 Fish husbandry

Atlantic salmon smolts were reared at Marine Harvest Freshwater Farms Hatchery in Duncan, British Columbia, Canada as described in Chapter 2. During the spawning season (November/December 2013), 291 mating families were established with the Scottish McConnell strain and the Norwegian Mowi strain, as well as hybrid crosses between the two strains. After fertilization, 19 fish eggs from each family were randomly selected and kept in a Heath tray; a total number of eggs was 5529. At the fry stage (April 2014), all the fish from the heath tray were moved into a 9,300-l aquarium where they were reared in flow-through well-water and using 24 h light until June 21, 2014, when they were switched to ambient light starting on. Temperature ranged naturally from 10 to 16 °C year-round. An automatic feeder was used to feed fish with a daily feed ration of 1 to 1.2% of body mass. Oxygen saturation was maintained between 80% and 120% (a Point Four System monitor dissolved oxygen concentration continuously). After reaching a smolt stage, the fish were moved into a 35,000-l aquarium. The smolt stage was identified using real-time PCR to detect the expression of NKAα1b (Na^+/K^+-ATPase α-1b isoform), which is the major isoform expressed during smolt development and used for seawater adaption (McCormick et al., 2013). Mean body mass of the smolts at the time of the hypoxia tolerance test (May 2015) was 220.2 ± 44.9 g and the mean fork length was 27.0± 2.9 cm.

3.2.2 Phenotyping: hypoxia tolerance

To test hypoxia tolerance, 40-50 fish were haphazardly selected from the rearing tank and transferred to a test aquarium (around 700 l). Bubble wrap was used to seal the surface of water

so that oxygen in the air could not diffuse rapidly into the water. Fish were allowed to adjust to the new environment for 30 min during which dissolved oxygen in the experimental tank was maintained above 80% by bubbling oxygen into the experimental tank. A submersible pump was used to mix the water in the experimental tank and an oxygen meter (OxyGuard Handy Polaris) was used to monitor oxygen saturation in the water. After this initial period, nitrogen was introduced into the water to decrease dissolved oxygen at a rate of 2% per minute until oxygen reached 20% of air saturation (2.1 mg/L DO). A timer was started once dissolved oxygen reached 20% of air saturation and the time at which individual fish lost equilibrium (LOE) was recorded. Fish that lost equilibrium were immediately removed from the tank and stunned by percussion before body mass, fork length and sex were measured. Fin clips were collected and stored in 95% ethanol for extracting DNA. A total of 855 Atlantic salmon smolts were tested for hypoxia tolerance using 18 separate trials over 10 days.

3.2.3 Genotyping and GWAS

The same approach as described in Chapter 2 was used to identify single nucleotide polymorphisms (SNPs) in Atlantic salmon smolts. In this case, the dataset retained 23,558 SNPs that were used for association analyses. GWAS was performed as detailed in Chapter 2. Briefly, a mixed linear model (MLM) in Tassel version 5.0 including population structure and family relatedness was used to carry out genome-wide association studies.

3.3 Results

3.3.1 Hypoxia tolerance

A wide range of variation in hypoxia tolerance was observed among individuals in Atlantic salmon smolts. The first fish lost equilibrium at 4.5 min before the target DO (2.1 mg/L) was reached, whereas the last fish did not lose equilibrium until 355.4 min at 2.1 mg/L (Fig. 3.1). Approximately 72% of smolts lost equilibrium within 30 min of hypoxia exposure and only 11% of smolts tolerated more than 60 min of hypoxia. Therefore, the majority of smolts were considered hypoxia-sensitive and few individuals were hypoxia-tolerant.

3.3.2 Body mass

Body mass varied substantially among Atlantic salmon smolts, ranging from 18 to 468 g, with a median of 212 g (Fig. 3.2).

3.3.2 Genotyping by sequencing

A total of 172 samples and 23,558 SNPs in the dataset passed the quality control and were used for association analyses. The number of SNPs per chromosome ranged from 218 on chromosome 8 (NC_027307.1) to 1,657 on chromosome 1 (NC_027300.1) (Fig. 3.3). An average of 10 SNPs per megabase (Mb) was identified over the entire genome, and this proportion ranged from 8 to 12 SNPs per megabase, depending on the chromosome (Fig. 3.3).

3.3.3 GWAS for hypoxia tolerance in smolts

2 SNPs were identified as significantly associated with hypoxia tolerance in smolts (Fig. 3.4A). The most significant SNP (S1_1107059674) was significantly associated with hypoxia

tolerance when a genome-wide FDR correction was applied (P = 1.79e-06), and the second significant SNP (S1_281926376) was associated with hypoxia tolerance when a chromosome-wide FDR correction was applied (P = 1.73e-05). SNP S1_1107059674 was located nearby the gene "regulator of G-protein signaling 17-like" on chromosome 13 (NC_027312.1). SNP S1_281926376 was located in the gene "ATP-sensitive inward rectifier potassium channel 12-like" on chromosome 3 (NC_027302.1) (Table 3.1). Comparing these SNPs to those identified as associated with hypoxia tolerance in Atlantic salmon adults (Chapter 2), there was no overlap between the SNPs identified at these two life-stages.

I also performed a GWAS for body mass in Atlantic salmon smolts. A total of sixty SNPs was identified as significantly associated with body mass in smolts (Figure 3.4B). The top two SNPs (S1_1823983926 and S1_1823983948) were significantly associated with body mass with genome-wide FDR correction (P = 2.97e-06 and P = 3.24e-06). These two SNPs were only 22 bp apart and located nearby the gene "ribulose-phosphate 3-epimerase-like" on chromosome 21 (NC_027320.1). The other fifty-eight SNPs were significantly associated with body mass when a chromosome-wide FDR correction was applied, and included fifty-five SNPs on chromosome 21 and three SNPs on chromosome 18 (NC_027317.1). The genes that these significant SNPs were located in or nearby were shown in Table 3.1. Comparing these SNPs to those identified as associated with body mass in adults (Chapter 2), there was no overlap in the SNPs identified as associated with body mass at these two life stages.

3.4 Discussion

In the present study, I conducted a GWAS to identify genetic variants associated with hypoxia tolerance in Atlantic salmon smolts using high-density SNP markers. Our goals were to

characterize the variation in hypoxia tolerance in smolts, identify the genetic basis of variation in hypoxia tolerance in smolts and determine whether there are common SNPs associated with hypoxia tolerance between smolts and adults. The three major findings were that: i) substantial variation in hypoxia tolerance exists among individuals in Atlantic salmon smolts; (ii) one SNP at the genome-wide level of significance (S1_1107059674) and one SNP at the chromosome-wide level of significance (S1_281926376) were associated with hypoxia tolerance in smolts; (iii) the SNPs associated with hypoxia tolerance differed between smolts and adults; and (iv) two SNPs at the genome-wide level of significance and fifty-eight SNPs at the chromosome-wide level of significance were identified associated with body mass, and these SNPs also differed from those associated with body mass in adults.

3.4.1 Hypoxia tolerance

A similar pattern of variation in hypoxia tolerance was observed in Atlantic salmon smolts, when compared to Atlantic salmon adults in the previous chapter. However, smolts exhibited a much wider range of variation in hypoxia tolerance than adults. High variation in hypoxia tolerance among Atlantic salmon smolts has also been reported by Barnes et al. (2011) who found that the PO_2 at which loss of equilibrium (LOE) occurred ranged from 1.76 mg/L to 3.17 mg/L at 14 °C in a Tasmanian population of Atlantic salmon (Barnes et al., 2011).

3.4.2 GWAS for hypoxia tolerance

Our GWAS was able to identify two SNPs (S1_1107059674 and S1_281926376) significantly associated with hypoxia tolerance in Atlantic salmon smolts. The most significant SNP, S1_1107059674, was closest to a gene coding for regulator of G-protein signaling 17-like

(RGS17) which is a member of regulator of G protein signaling (RGS) gene family. Members of this gene family are responsible for rapidly turning off the G protein-coupled receptor (GPCR) signaling pathway (Vries et al., 2000). The GPCR signaling pathway has been widely studied and plays a very important role in cellular responses to extracellular stimuli in both the short term and long term (Vries et al., 2000). GPCR family members are thought to be important in the response of cells to hypoxic conditions (Lappano et al., 2016). For example, G-proteins have been identified to playing an important role in cellular responses to hypoxia in guinea pig heart (Xu et al., 1993). In fish, significant changes in G-protein expression has been detected in response to hypoxia in two northern populations of European flounder during two months of warm and hypoxic exposure (Pédron et al., 2017).

The second significant SNP, S1_281926376, that was significantly associated with variation in hypoxia tolerance was located in the gene ATP-sensitive inward rectifier potassium (K_{ATP}) channel 12-like. K_{ATP} channels are members of the inwardly rectifying K^+ (Kir) superfamily (Noma, 1983) and are found in many tissues including muscle, brain, heart and pancreatic beta cells (Noma, 1983; Sun & Feng, 2013). Their activity is regulated by adenine nucleotides and activated when the ATP/ADP ratio decreases. Thus, their function is tightly linked to cellular metabolism (Tinker et al., 2014). Moreover, K_{ATP} channels have been repeated shown to be associated with a protective role during hypoxia in a wide range of tissues and species. For example, Noma (1983) showed that hypoxia triggered an outward K^+ current in heart muscle cells, but this was reversed after ATP was injected into the cell. Neuronal K_{ATP} was suggested to play a protective role in brain hypoxia (Ballanyi, 2004). Many studies have shown that K_{ATP} channels are associated with hypoxia tolerance in gold fish. For example, the activities of K_{ATP} channel 8 and channel 1 increased significantly in ventricles during the chronic hypoxia

(Cameron et al., 2013); hypoxia-induced activities of sarcolemma K_{ATP} and mitochondria K_{ATP} channels in cardiac muscle cells were suggested to enhance hypoxia tolerance during hypoxic exposure (Chen et al., 2005).

3.4.3 GWAS for body mass

Sixty SNPs were identified to be significantly associated with body mass in Atlantic salmon smolts. These SNPs included two SNPs that were significantly associated with body mass when genome-wide FDR correction was applied and fifty-eight SNPs that were significantly associated with body mass when chromosome-wide FDR correction was applied. At the genome-wide level of significance, the two detected SNPs (S1_1823983926 and S1_1823983948) were only 22 bp apart and located nearby ribulose-phosphate 3-epimerase-like (RPE). RPE is an enzyme that catalyzes the reversible reaction from D-ribulose 5-phosphate to D-xylulose 5-phosphate (Liang et al., 2011). This reaction drives the nonoxidative phase of the pentose phosphate pathway (PPP), which supplies the majority of the NADPH for the biosynthetic purposes in humans (Wood, 1986). Previously, rainbow trout exhibited regulation of the oxidative phase of the pentose phosphate pathway during starvation and refeeding, and the pathway in the muscle of re-feeding fish was suggested to produce nucleotides and nucleic acids to improve the DNA and RNA synthesis during periods of rapid growth (Johansen & Overturf, 2006). A similar reaction might occur in the non-oxidative phase of the pentose phosphate pathway.

At the chromosome-wide level of significance, many interesting genes that these significant SNPs located in or nearby included ephrin-B2, NGFI-A-binding protein 1-like, low-density lipoprotein receptor-related protein 1B-like, alpha/beta hydrolase domain-containing

protein 13, diacylglycerol kinase eta-like, and transcription factor Sp3-like (Table 3.1). SNP S1_1812998013 and SNP S1_1812790438 are closest to a gene coding for ephrin-B2 which is a transmembrane ligand for Eph receptor tyrosine kinases. Ephrin-B2 has been reported to play a key role in vascular endothelial growth factor (VEGF) pathway and control angiogenic and lymphangiogenic growth in zebrafish and mouse (Wang et al., 2010). Hypoxia acclimation of rainbow trout has recently been shown to increase oxygen uptake at tissues likely through increased capillarization (Motyka et al., 2016). SNP S1_1814615966 is located around 1 kb upstream of NGFI-A-binding protein 1-like, also known as early growth response protein-1 (Egr-1). NGFI-A family proteins have been suggested to be involved in the genetic regulation of cell growth in response to extracellular stimuli (Gashler & Sukhatme, 1995). Seven SNPs (S1_1834996035, S1_1834879424, S1_1835146241, S1_1835146244, S1_1835146245, S1_1835599062, S1_1835026048) are located within or nearby low-density lipoprotein (LDL) receptor-related protein 1B-like (LRP1), which belongs to a member of LDL receptor family that is involved in various biological functions including metabolising lipoproteins, activating lysosomal enzymes, and degrading proteases (Lillis et al., 2009). SNP S1_1822450359 is located nearby alpha/beta hydrolase domain (ABHD)-containing protein 13, which is a member of ABHD family that plays an important role in regulating lipid metabolism, lipid signal transduction and metabolic disease (Lord et al., 2013). SNP S1_1816526547 and SNP S1_1816526557 are located with diacylglycerol kinase (DGK) eta-like that catalyzes the phosphorylation of diacylglycerol to produce phosphatidic acid. This gene was considered very important in promoting cell growth (Yasuda et al., 2009). SNP S1_1811703406 is located nearby transcription factor Sp3-like that is a member of the specificity protein (Sp) transcription factor family. Transcription factor Sp3-like was identified to play an important role in regulating the

fibroblast growth factor receptor 1 (FGFR1) gene in skeletal muscle cells (Parakati & DiMario, 2002).

3.5 Conclusion

The goals of this study were to identify the genetic markers significantly associated with hypoxia tolerance in Atlantic salmon smolts and determine whether there are common genetic markers associated with hypoxia tolerance between smolts and adults. I identified one SNP using genome-wide FDR correction and one SNP using chromosome-wide FDR correction. Nevertheless, there are no common significant SNPs associated with hypoxia tolerance between smolts and adults. The significant SNPs that I detected in adults could potentially be used for Marine Harvest Canada to improve hypoxia tolerance in seawater through marker assisted selection. In addition, I identified two SNPs associated with body mass with genome-wide FDR correction and fifty-eight SNPs associated with body mass with chromosome-wide FDR correction.

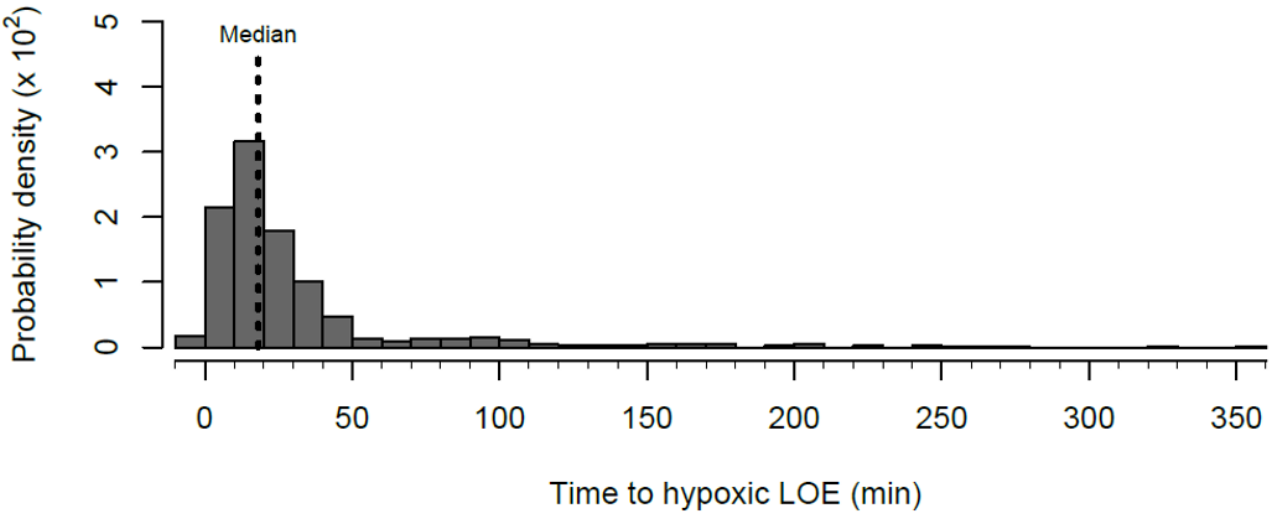

Figure 3.1The distribution of hypoxia tolerance in Atlantic salmon smolts in freshwater. Hypoxia tolerance was measured as time-to-loss of equilibrium at 2.1 mg/L oxygen. A total of 855 smolts in freshwater were tested for hypoxia tolerance. The median time-to-LOE was 18.1 min.

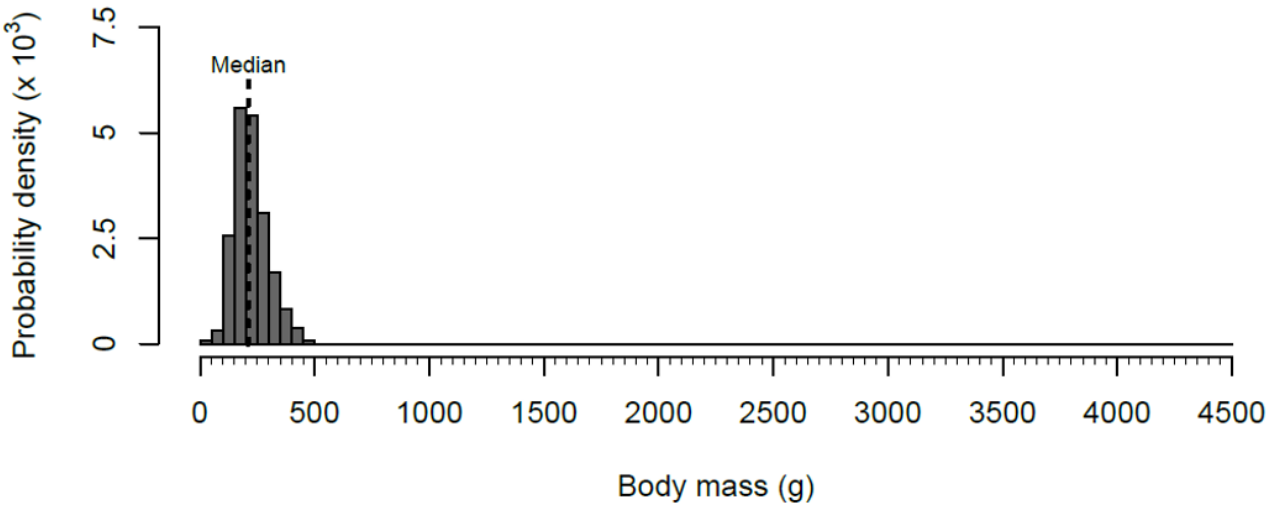

Figure 3.2 The distribution of body mass in Atlantic salmon smolts in freshwater. A total of 855 adults were measured for body mass and the median was 212 g.

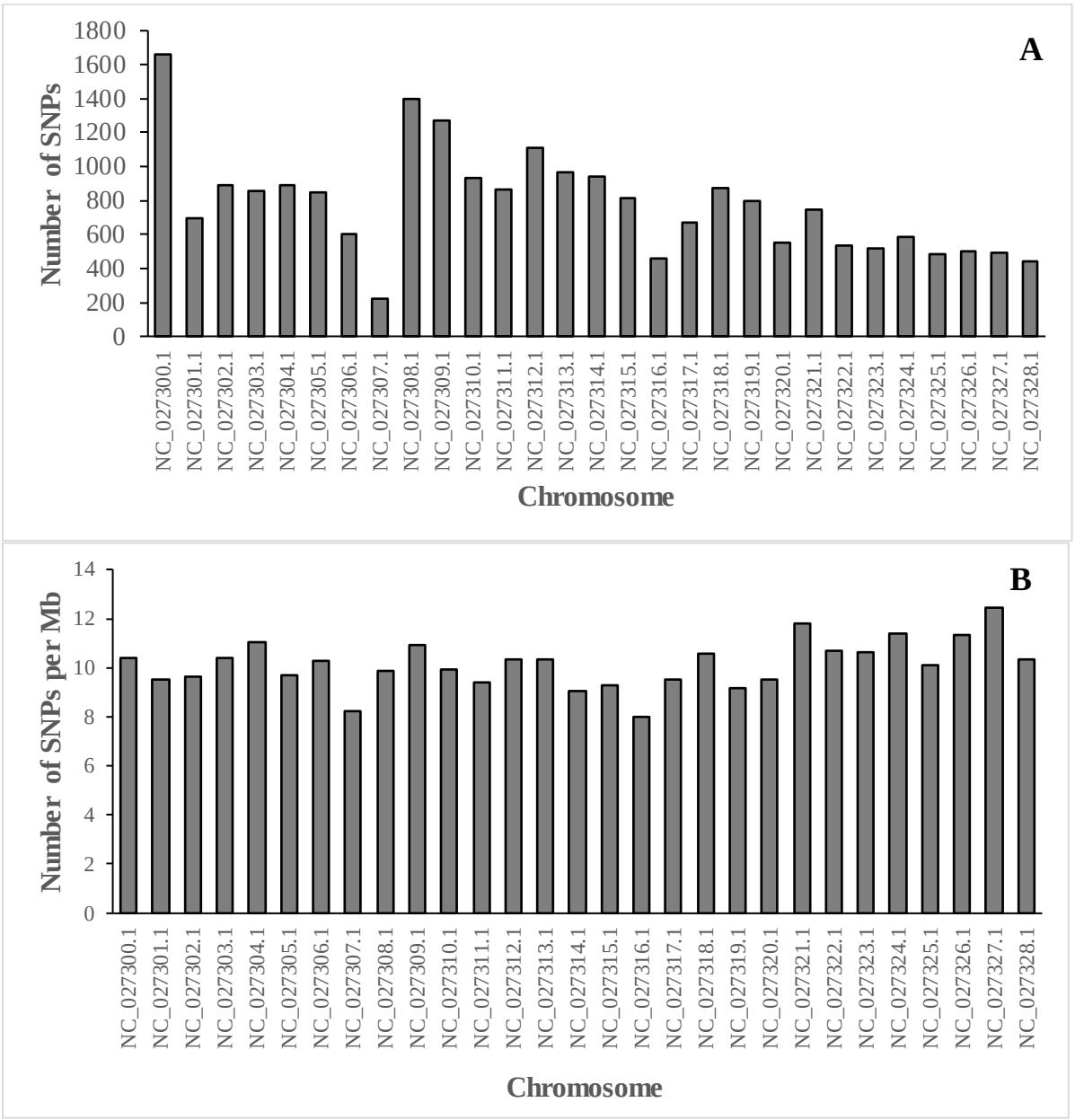

Figure 3.3 (A) The total number of SNPs identified across the Atlantic salmon genome and (B) the proportion of SNPs by chromosome calculated in SNPs/Mb.

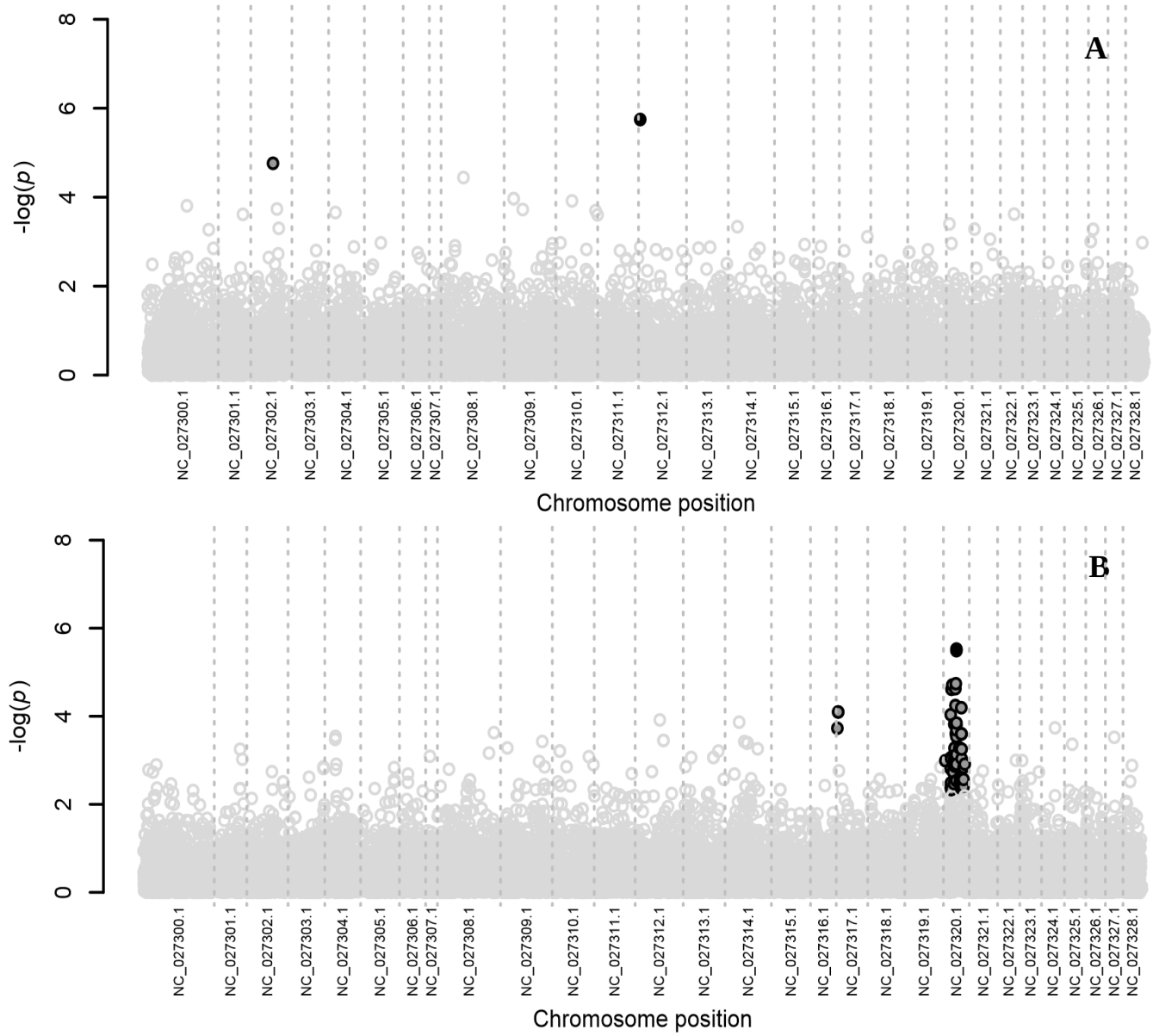

Figure 3.4 Manhattan plots displaying the significance of associations between each identified SNP and phenotypes in Atlantic salmon smolts. (A) hypoxia tolerance, (B) body mass. The black dots represent SNPs that are significantly associated with the specified trait with genome-wide FDR correction, and the grey dots indicate SNPs that are significantly associated with the specified trait with chromosome-wide FDR correction.

Table 3.1 Hypoxia tolerance and body mass associations identified in the analyses. Chromosome, alleles, genes, *P*-values and marker r^2 of significant SNPs at the genome level of significance and chromosome level of significance for hypoxia tolerance and body mass in Atlantic salmon smolts.

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Hypoxia	Genome	S1_1107059674	NC_027312.1	A/G	Nearest regulator of G-protein signaling 17-like	1.79e-06	0.17
Hypoxia	Chromosome	S1_281926376	NC_027302.1	A/G	ATP-sensitive inward rectifier potassium channel 12-like	1.73e-05	0.15
Body mass	Genome	S1_1823983926	NC_027320.1	A/T	Nearest (around 20 kb) ribulose-phosphate 3-epimerase-like	2.97e-06	0.14
Body mass	Genome	S1_1823983948	NC_027320.1	C/G	Nearest (around 20 kb) ribulose-phosphate 3-epimerase-like	3.24e-06	0.14
Body mass	Chromosome	S1_1812998013	NC_027320.1	G/T	Nearest (around 260 kb) ephrin B	2.44e-05	0.12
Body mass	Chromosome	S1_1814615966	NC_027320.1	C/T	Nearest (around 1 kb) NGFI-A-binding protein 1-like	1.95e-05	0.12
Body mass	Chromosome	S1_1821693955	NC_027320.1	A/T	Transcriptional regulator ATRX-like	2.36e-05	0.12
Body mass	Chromosome	S1_1822686150	NC_027320.1	A/G	Nearest (8 kb) transmembrane protein FAM155A-like	1.82e-05	0.12
Body mass	Chromosome	S1_1821697996	NC_027320.1	A/C	Transcriptional regulator ATRX-like	5.68e-05	0.12

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Body mass	Chromosome	S1_1834996035	NC_027320.1	A/G	Low-density lipoprotein receptor-related protein 1B-like	6.39e-05	0.11
Body mass	Chromosome	S1_1811204833	NC_027320.1	G/T	Glutamate decarboxylase 1-like	9.20e-05	0.11
Body mass	Chromosome	S1_1819101070	NC_027320.1	A/C	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A	1.52e-04	0.10
Body mass	Chromosome	S1_1823654532	NC_027320.1	A/-	Nearest (289 bp) chromatin assembly factor 1 subunit B-like	1.62e-04	0.10
Body mass	Chromosome	S1_1823654533	NC_027320.1	T/-	Nearest (290 bp) chromatin assembly factor 1 subunit B-like	1.62e-04	0.10
Body mass	Chromosome	S1_1824083245	NC_027320.1	C/G	Immunoglobulin superfamily member 3-like	1.42e-04	0.10
Body mass	Chromosome	S1_1822491846	NC_027320.1	C/T	Transmembrane protein FAM155A-like	2.48e-04	0.10
Body mass	Chromosome	S1_1823876421	NC_027320.1	A/G	Vang-like protein 1	2.04e-04	0.11
Body mass	Chromosome	S1_1834879424	NC_027320.1	A/G	Nearest (around 105 kb) low-density lipoprotein receptor-related protein 1B-like	2.51e-04	0.09
Body mass	Chromosome	S1_1835146241	NC_027320.1	G/T	Low-density lipoprotein receptor-related protein 1B-like	2.51e-04	0.09
Body mass	Chromosome	S1_1835146244	NC_027320.1	G/-	Low-density lipoprotein receptor-related protein 1B-like	2.51e-04	0.09

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Body mass	Chromosome	S1_1835146245	NC_027320.1	T/-	Low-density lipoprotein receptor-related protein 1B-like	2.51e-04	0.09
Body mass	Chromosome	S1_1823654427	NC_027320.1	G/T	Nearest (184 bp) uncharacterized LOC106582112	2.89e-04	0.10
Body mass	Chromosome	S1_1819911488	NC_027320.1	T/-	Interleukin-1 receptor accessory protein-like 1-B	5.29e-04	0.09
Body mass	Chromosome	S1_1819911529	NC_027320.1	C/-	Interleukin-1 receptor accessory protein-like 1-B	5.29e-04	0.09
Body mass	Chromosome	S1_1830431766	NC_027320.1	A/T	Nearest (around 9 kb) LON peptidase N-terminal domain and RING finger protein 2-like	5.17e-04	0.09
Body mass	Chromosome	S1_1835650191	NC_027320.1	C/T	Nearest (around 10 kb) ras-related protein Ral-B-like	5.57e-04	0.09
Body mass	Chromosome	S1_1823876359	NC_027320.1	C/G	Vang-like protein 1	7.42e-04	0.09
Body mass	Chromosome	S1_1811490640	NC_027320.1	A/G	Integrin alpha-6-like	8.71e-04	0.08
Body mass	Chromosome	S1_1812790438	NC_027320.1	G/T	Nearest (around 53 kb) ephrin B2	9.31e-04	0.08
Body mass	Chromosome	S1_1819260257	NC_027320.1	A/T	Rho guanine nucleotide exchange factor 7-like	9.51e-04	0.08
Body mass	Chromosome	S1_1835599062	NC_027320.1	A/G	Nearest (around 4.7 kb) low-density lipoprotein receptor-related protein 1B-like	9.17e-04	0.08

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Body mass	Chromosome	S1_1798969437	NC_027320.1	A/C	Integrin subunit beta like 1	1.02e-03	0.08
Body mass	Chromosome	S1_1822450359	NC_027320.1	A/T	Nearest (around 37 kb) alpha/beta hydrolase domain-containing protein 13	1.06e-03	0.08
Body mass	Chromosome	S1_1823241840	NC_027320.1	A/T	Follistatin like 1	1.04e-03	0.08
Body mass	Chromosome	S1_1817463999	NC_027320.1	C/T	G protein-coupled receptor 34	1.26e-03	0.08
Body mass	Chromosome	S1_1824083377	NC_027320.1	A/T	Immunoglobulin superfamily member 3-like	1.23e-03	0.08
Body mass	Chromosome	S1_1842733569	NC_027320.1	A/G	Nearest (around 105 kb) CMRF35-like molecule 8	1.22e-03	0.08
Body mass	Chromosome	S1_1820085653	NC_027320.1	C/T	Interleukin-1 receptor accessory protein-like 1-B	1.36e-03	0.08
Body mass	Chromosome	S1_1840055969	NC_027320.1	C/-	Transmembrane protein 177	1.45e-03	0.08
Body mass	Chromosome	S1_1811254731	NC_027320.1	C/T	Tousled like kinase 1	1.56e-03	0.08
Body mass	Chromosome	S1_1811254739	NC_027320.1	A/T	Tousled like kinase 1	1.56e-03	0.08
Body mass	Chromosome	S1_1816526547	NC_027320.1	A/-	Diacylglycerol kinase eta-like	1.83e-03	0.07

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Body mass	Chromosome	S1_1816526557	NC_027320.1	C/-	Diacylglycerol kinase eta-like	1.83e-03	0.07
Body mass	Chromosome	S1_1558676110	NC_027317.1	A/C	Prominin-1-A-like	7.99e-05	0.11
Body mass	Chromosome	S1_1558676111	NC_027317.1	A/C	Prominin-1-A-like	7.99e-05	0.11
Body mass	Chromosome	S1_1832039976	NC_027320.1	A/G	Nearest (around 95 kb) muscle M-line assembly protein unc-89-like	2.44e-03	0.07
Body mass	Chromosome	S1_1835026048	NC_027320.1	C/T	Low-density lipoprotein receptor-related protein 1B-like	2.71e-03	0.07
Body mass	Chromosome	S1_1836662262	NC_027320.1	C/G	Zinc finger protein GLI2-like	2.67e-03	0.07
Body mass	Chromosome	S1_1840055970	NC_027320.1	A/-	Transmembrane protein 177	2.71e-03	0.07
Body mass	Chromosome	S1_1832923046	NC_027320.1	C/T	Erb-b2 receptor tyrosine kinase 4	2.78e-03	0.07
Body mass	Chromosome	S1_1823704344	NC_027320.1	A/T	MORC family CW-type zinc finger protein 3-like	2.90e-03	0.07
Body mass	Chromosome	S1_1811703406	NC_027320.1	C/T	Nearest (around 14 kb) transcription factor Sp3-like	3.25e-03	0.07
Body mass	Chromosome	S1_1831120847	NC_027320.1	A/C	Integrin alpha-4-like	3.31e-03	0.07

Trait	Significance Level	Marker	Chromosome	Alleles	Genes	*P*-values	Marker r^2
Body mass	Chromosome	S1_1818497835	NC_027320.1	A/C	Nearest (around 4 kb) extensin-like	3.38e-03	0.07
Body mass	Chromosome	S1_1556855939	NC_027317.1	A/C	Arginyltransferase 1	1.87e-04	0.10
Body mass	Chromosome	S1_1834258153	NC_027320.1	C/T	Nearest (around 14 kb) GDP-fucose protein O-fucosyltransferase 2-like	4.04e-03	0.07
Body mass	Chromosome	S1_1835653001	NC_027320.1	A/G	Nearest (around 7 kb) ras-related protein Ral-B-like	4.07e-03	0.06
Body mass	Chromosome	S1_1840055968	NC_027320.1	A/-	Transmembrane protein 177	3.96e-03	0.06
Body mass	Chromosome	S1_1811204761	NC_027320.1	C/G	Glutamate decarboxylase 1-like	4.21e-03	0.07
Body mass	Chromosome	S1_1834405565	NC_027320.1	C/T	Unconventional myosin-Ib-like	4.30e-03	0.06
Body mass	Chromosome	S1_1837616473	NC_027320.1	A/C	Nearest (around 31 kb) integrin beta-5-like	4.40e-03	0.06
Body mass	Chromosome	S1_1812626170	NC_027320.1	A/C	Nearest (around 49 kb) CD276 antigen homolog	4.63e-03	0.07

Chapter 4. General Discussion

In this thesis, I performed a GWAS for hypoxia tolerance and body size in Atlantic salmon to improve our understanding of the factors determining the ability of fish to tolerate hypoxia. Episodes of hypoxia are increasing in frequency in British Columbia, and thus these data are likely to be useful to aquaculture producers such as our partners in this research, Marine Harvest Canada, that produce Atlantic salmon in sea cages. In this chapter, I provide a brief summary of my results from the GWAS for hypoxia tolerance and body size, outline some strengths and limitations of my research, and discuss the implications of my findings.

4.1 Summary of results for hypoxia tolerance

4.1.1 GWAS for hypoxia tolerance in Atlantic salmon

I characterized variation in hypoxia tolerance and identified a potential genetic basis of this variation in Atlantic salmon at two life stages: adults in seawater and smolts in freshwater just prior to seawater transfer. At both life stages, I observed substantial variation in hypoxia tolerance. In adults in seawater, time-to-LOE at 2.1 mg/L DO ranged from 4.6 min to 126.9 min. Moreover, I found that strain had an effect on hypoxia tolerance, with McConnell strain having better hypoxia tolerance than Mowi strain. A similar pattern but wider range of variation in hypoxia tolerance was observed in smolts, with time-to-LOE at 2.1 mg/L DO ranging from 4.5 min before the target DO was reached to 355.4 min in hypoxia. However, there was no significant difference in overall hypoxia tolerance between the two life stages, and median time-to-LOE was 16.0 min in adults and 18.1 min in smolts.

Through GWAS, I identified two SNPs that were associated with hypoxia tolerance using genome-wide FDR correction and six SNPs using chromosome-wide FDR correction in adults and one SNP using genome-wide FDR correction and one SNP using chromosome-wide FDR correction in smolts.

However, there was no overlap in the SNPs identified as associated with hypoxia tolerance at these two life stages. Four out of eight significant SNPs that were associated with hypoxia tolerance in adults in seawater (S1_982279883, S1_982279912, S1_982279912 and S1_871007038) were bit sequenced in freshwater dataset, and the other four significant SNPs were present in the smolts in freshwater but were not significantly associated with hypoxia tolerance (S1_871678299, S1_871678299, S1_871872804 and S1_867269825). The most significant SNP (S1_1820911774) associated with hypoxia tolerance in smolts in freshwater was not sequenced in seawater dataset, and the second significant SNP (S1_1107059674) was present but was not significantly associated with hypoxia tolerance in adults.

4.1.2 Factors affecting hypoxia tolerance

Body mass is a factor that has the potential to affect hypoxia tolerance. For example, body mass is positively correlated with hypoxia tolerance in channel catfish (*Ictalurus punctatus*) (Wang et al., 2017). Similarly, in the Oscar cichlid (*Astronotus ocellatus*) of the Amazon River, large individuals had better hypoxia tolerance than smaller ones (Almeida-Val et al., 2000). In contrast, in salmonids Anttila et al. (2013) found that there was no correlation between body mass and time-to-LOE in the St. John strain of Atlantic salmon and previous work in rainbow trout (*Onchorynchus mykiss*), another salmonid species, has demonstrated that body mass does not affect time-to-LOE (Scott et al., 2014). Nevertheless, in this study I found that there was a

significant negative correlation between time-to-LOE and body mass in both smolts ($y = -0.0598x + 44.263, r^2 = 0.011, P < 0.0001$) and adults ($y = -0.0062x + 34.242, r^2 = 0.051, P = 0.0022$), but only 1.1% and 5.1% of variation of these data were explained respectively. However, this did not result in a clear difference in hypoxia tolerance between smolts and adults.

Salinity is another potential factor that can affect hypoxia tolerance. In a meta-analysis of published studies, Rogers et al. (2016) showed that salinity is an important predictor of inter-specific variation in hypoxia tolerance. This observation is consistent with osmoregulatory theory, which suggests that the metabolic cost of osmoregulation in fish increases when salinity of water deviates from isosmotic (Fry, 1971). However, when comparisons are made within species, the salinity effect on hypoxia tolerance varies substantially among different species, with some fish showing no salinity effects and others exhibiting large effects (Febry & Lutz, 1987). In my experiments, I did not detect any difference in overall hypoxia tolerance between Atlantic salmon smolts in freshwater and adults in seawater, suggesting that salinity has a limited effect on hypoxia tolerance in this species.

Life stage is another potential factor affecting hypoxia tolerance when comparing smolts to adults. Some studies have shown that life stage is correlated with hypoxia tolerance. For example, P_{crit} in steelhead trout (*Oncorhynchus mykiss*) increased with age during the embryonic stage (Rombough, 1988). However, no other studies have been done to compare hypoxia tolerance among different life stages in Atlantic salmon. In particular, in my study I compared adults in seawater to smolts in freshwater just prior to the normal time of seawater transfer. Smoltification is an energetically expensive process that involves substantial remodeling of the

gill (Tocher et al., 2000) that might be expected to affect hypoxia tolerance. However, a difference in hypoxia tolerance was not observed between smolts and adults in this study, suggesting that these factors either do not have effects on hypoxia tolerance or have several opposite effects on hypoxia tolerance that cancel each other out.

4.2 Summary of results for body mass

In both Atlantic salmon adults and smolts, I observed a wide range of variation in body mass. Body mass ranged from 18 g to 468 g in smolts and 213 to 4,260 g in adults. In adults, a significant difference was identified in body mass at the family level but not at strain level. Through GWAS, I identified four significant SNPs associated with body mass in adults using chromosome-wide FDR correction, and in smolts I identified two SNPs associated with body mass with genome-wide FDR correction and thirteen SNPs associated with body mass with chromosome-wide FDR correction. However, there was no overlap in the SNPs identified as associated with body mass at these two life stages. In the seawater dataset, the four significant SNPs (S1_1570467139, S1_224141594, S1_179657087, S1_204440772) existed in freshwater dataset but were not significantly associated with body mass. In the freshwater dataset, the 15 significant SNPs existed in the seawater dataset but were not significantly associated with body mass. Previous GWAS have identified different genes that could be potentially associated with growth trait in Atlantic salmon. For example, a hypothetical protein in the vicinity of a membrane-associated guanylate kinase protein was identified by Gutierrez et al. (2015); the PCNT and MEP1A genes were detected by Tsai et al. (2015); the retinoic acid-induced protein gene (RAI2), protein-o-mannosyltransferase 1 (POMT1), agrin (AGRN), notch 3 (NOTCH3), myosin (MYH9) and MYO18AB were identified by Tsai et al. (2015).

4.3 Strengths and limitations of this research

The primary strength of the research presented in this thesis is that I examined variation in hypoxia tolerance at two life-stages of Atlantic salmon. These fish were from a single brood year, and thus the data generated across these two life stages are directly comparable. I found that hypoxia tolerance was not significantly different between these two life stages, but that there was substantial variation in hypoxia tolerance among individuals. In addition, I demonstrated that there is variation in hypoxia tolerance among strains of Atlantic salmon, which suggests that there could be a genetic basis of this phenotype.

The main limitation of my research is the relatively small sample size used for GWAS. My study used 172 smolts and 185 adults for two separate GWAS, whereas a much higher number of individuals have been used for previous GWAS in Atlantic salmon. For example, 466 individuals were used to identify the genetic basis of variation in growth and age at sexual maturation in Atlantic salmon (Gutierrez, 2015); 2,391 individuals were used to identify the association between SNPs and resistance against *Piscirickettsia salmonis* in Atlantic salmon (Correa et al., 2015).

4.4 Potential implication for selective breeding and future directions

In my thesis I detected a small number of significant SNPs associated with either hypoxia tolerance or body mass in Atlantic salmon. There was no overlap between the SNPs identified for hypoxia tolerance and body mass, which indicates that hypoxia-tolerant fish could be potentially selected through marker-assisted selection (MAS) without compromising growth in Atlantic salmon. Importantly, there was no overlap in the SNPs identified as associated with hypoxia tolerance at smolt and adult stages, this suggests that hypoxia tolerance in freshwater is

unlikely to predict performance in hypoxia in seawater. However, the significant SNPs that I detected in adults could potentially be used for Marine Harvest Canada to improve hypoxia tolerance in seawater through marker-assisted selection. The potentially important markers identified in this study should be followed up with larger sample sizes to confirm whether the associations detected here are robust.

A combination of GWAS and quantitative trait locus (QTL) mapping is another way to improve the power to identify true genotype-phenotype associations (Korte & Farlow, 2013). Compared to QTL mapping, GWAS overcomes the limitations associated with the lack of recombination events (Korte & Farlow, 2013; Nordborg & Tavaré, 2002). However, unlike QTL mapping, GWAS has lower power for detection and is prone to identify false-positives (Aranzana et al., 2005; Korte & Farlow, 2013). Therefore, GWAS and QTL mapping are complementary and a combination of these two approaches can mitigate each other's limitations (Korte & Farlow, 2013; Zhao et al., 2007). Thus, a combined GWAS and QTL study for hypoxia tolerance could potentially reveal additional markers that could be associated with hypoxia tolerance.

References

Almeida-Val, V.M.F., Val, A.L, Duncan, W.P., Souza, F.C.A., Paula-Silva, M.N., & Land, S. (2000). Scaling effects of hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes: Cichlidae): contribution of tissue enzyme levels. *Comparative Biochemistry and Physiology, 125B*, 129-226.

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., … Schulte, P. M. (2013). Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *The Journal of Experimental Biology*, *216*(Pt 7), 1183–1190.

Aranzana, M. J., Kim, S., Zhao, K., Bakker, E., Horton, M., Jakob, K., … Nordborg, M. (2005). Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. *PLoS Genetics*, *1*(5).

Ashkov, S. (1979). Adenomectomy and herniotomy at the same time. *Khirurgiia*, *32*(6), 547–51.

Azanza, R. V, Fukuyo, Y., Yap, L. G., & Takayama, H. (2005). Prorocentrum minimum bloom and its possible link to a massive fish kill in Bolinao, Pangasinan, Northern Philippines. *Harmful Algae*, *4*(3), 519–524.

Bailey, G. S., Poulter, R. T. M., & Stockwell, P. A. (1978). Gene duplication in tetraploid fish: Model for gene silencing at unlinked duplicated loci. *Proceedings of the National Academy of Sciences*, *75*(11), 5575–5579.

Ballanyi, K. (2004). Protective role of neuronal KATP channels in brain hypoxia. *Journal of Experimental Biology*, *207*(18), 3201–3212.

Baregamian, N., Rychahou, P.G., Hawkins, H.K., Evers, M., & Chung, D.H. (2007). Phosphatidylinositol 3-kinase pathway regulates hypoxia-inducible factor-1 to pretect from intestinal injury during necrotizing enterocolitis. *Surgery*, *142*(2), 295-302.

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*(3–4), 397–401.

Begum, H., Spindel, J. E., Lalusin, A., Borromeo, T., Gregorio, G., Hernandez, J., … McCouch, S. R. (2015). Genome-wide association mapping for yield and other agronomic traits in an elite breeding population of tropical rice (*Oryza sativa*). *PLoS ONE*, *10*(3), 1–19.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 289-300.

Blucher, A. (2015). Petuna says salmon killed in storm a "one-off".

Boesch, D. F. (2002). Challenges and opportunities for science in reducing nutrient over-enrichment of coastal ecosystems. *Estuaries*, *25*(4), 886–900.

Bond, P. (2016). Regulation of mTORC1 by growth factors, energy status, amino acids and mechanical stimuli at a glance. *Journal of the International Society of Sports Nutrition*, *13*(1), 8.

Bouchet, V. M. P., Debenay, J.-P. P., Sauriau, P.-G. G., Radford-Knoery, J., & Soletchnik, P. (2007). Effects of short-term environmental disturbances on living benthic foraminifera during the Pacific oyster summer mortality in the Marennes-Oléron Bay (France). *Marine Environmental Research*, *64*(3), 358–383.

Boutilier, R. G., & St-Pierre, J. (2000). Surviving hypoxia without really dying. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *126*(4), 481–490.

Braaten, B. R. (2007). Cage aquaculture and environmental impacts. *Aquacultural engineering and environment*, *661*(2), 49-91 .

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics (Oxford, England)*, *23*(19), 2633–2635.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789.

Buentello, J. a, Gatlin III, D. M., & Neill, W. H. (2000). Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish (*Ictalurus punctatus*). *Aquaculture*, *182*, 339–352.

Burt, K., Hamoutene, D., Mabrouk, G., Lang, C., Puestow, T., Drover, D., … Page, F. (2012). Environmental conditions and occurrence of hypoxia within production cages of Atlantic salmon on the south coast of Newfoundland. *Aquaculture Research*, *43*(4), 607–620.

Cameron, J.S., DeWitt, J.P., Ngo, T.T, Yajnik, T., Chan, S., Chung, E., & Kang, E. (2013). Cardiac K_{ATP} channel alterations with acclimation to hypoxia in goldfish (*Carassius auratus* L.). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *164*(4), 554-564.

Campbell, C. D., Ogburn, E. L., Lunetta, K. L., Lyon, H. N., Freedman, M. L., Groop, L. C., … Hirschhorn, J. N. (2005). Demonstrating stratification in a European American population. *Nature Genetics*, *37*(8), 868–872.

Chapman, L. ., & Mckenzie, D. (2009). Behavioral responses and ecological consequences. *Fish Physiology*, *27*, 25–77.

Chapman, L. J., Chapman, C. A., Brazeau, D. A., McLaughlin, B., & Jordan, M. (1999). Papyrus swamps, hypoxia, and faunal diversification: variation among populations of *Barbus neumayeri*. *Journal of Fish Biology*, *54*(2), 310–327.

Chen, J., Zhu, J.X., Wilson, I. and Cameron, J.S., (2005). Cardioprotective effects of KATP channel activation during hypoxia in goldfish *Carassius auratus*. *Journal of experimental biology*, *208*(14), 2765-2772.

Claireaux, G., Théron, M., Prineau, M., Dussauze, M., Merlin, F. X., & Le Floch, S. (2013). Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, *Dicentrarchus labrax*. *Aquatic Toxicology*, *130–131*, 160–170.

Correa, K., Lhorente, J. P., López, M. E., Bassini, L., Naswa, S., Deeb, N., … Yáñez, J. M. (2015). Genome-wide association analysis reveals loci associated with resistance against Piscirickettsia salmonis in two Atlantic salmon (*Salmo salar* L.) chromosomes. *BMC Genomics*, *16*(1), 854.

Davis, J. C. (1975). Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *Journal of the Fisheries Board of Canada*, *32*(12), 2295–2332.

Diaz, R. J. (2001). Overview of hypoxia around the world. *Journal of Environmental Quality*, *30*(2), 275–281.

Diaz, R. J., & Breitburg, D. L. (2009). The hypoxic environment. *Fish Physiology*, *27*, 1–23.

Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine biology. An Annual Review*, *33*, 203–245.

Doudoroff, P., & Shumway, D. L. (1970). Bibliographie des ouvrages et articles de Pierre Pascal. *Revue Des Études Slaves*, *54*(86), 255–270.

Dunn, J., & Hochachka, P. (1986). Metabolic responses of trout (*Salmo gairdneri*) to acute environmental hypoxia. *Journal of Experimental Biology*, *242*, 229–242.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS One*, *6*(5), e19379.

FAO. (2016). The State of World Fisheries and Aquaculture (SOFIA).

FAO. (2012). Yearbook 2010: Fishery and Aquaculture Statistics. *Food and Agriculture Organisation of the United Nations, Rome*, *78*.

Farrell, A. P. (2007). Tribute to P. L. Lutz: a message from the heart--why hypoxic bradycardia in fishes? *The Journal of Experimental Biology*, *210*(Pt 10), 1715–1725.

Farrell, A. P., & Richards, J. G. (2009). Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. *Fish Physiology*, *27*, 487–503.

Febry, R., & Lutz, P. (1987). Energy partitioning in fish : the activity- related cost of osmoregulation in a euryhaline cichlid. *Journal of Experimental Biology*, *128*, 63–85.

Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, *17*(4), 581–613.

Fry, F. E. J. (1971). 1 – The effect of environmental factors on the physiology of fish. In *Fish Physiology*, *6*, 1–98.

Gamperl, A. K., & Driedzic, W. R. (2009). Cardiovascular function and cardiac metabolism. *Fish Physiology*, *27*, 301-360.

Gamperl, A. K., Vijayan, M. M., & Boutilier, R. G. (1994). Experimental control of stress hormone levels in fishes: techniques and applications. *Reviews in Fish Biology and Fisheries*, *4*(2), 215–255.

Geist, D. R., Abernethy, C. S., Hand, K. D., Cullinan, V. I., Chandler, J. A., & Groves, P. A. (2006). Survival, development, and growth of fall Chinook salmon embryos, alevins, and fry exposed to variable thermal and dissolved oxygen regimes. *Transactions of the American Fisheries Society*, *135*(6), 1462–1477.

Gashler, A., Sukhatme, V.P. (1995). Early growth response protein 1 (Egr-1): prototype of a zinc finger family of transcription factors. *Progress in Nuvleic Acid Research and Molecular Biology*, *50*, 191-224.

Ginneken Van, V., van Den Thillart, G., Muller, H., van Deursen, S., Onderwater, M., Visee, J., … Nicolay, K. (1999). Phosphorylation state of red and white muscle in tilapia during graded hypoxia: an in vivo (31)P-NMR study. *The American Journal of Physiology*, *277*, R1501-12.

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS ONE*, *9*(2).

Graham, J. B. (1997). Air-breathing fishes: evolution, diversity, and adaptation. Academic Press.

Grantham, B. A., Chan, F., Nielsen, K. J., Fox, D. S., Barth, J. A., Huyer, A., … Menge, B. A. (2004). Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature*, *429*, 749–754.

Gudbjartsson, D. F., Walters, G. B., Thorleifsson, G., Stefansson, H., Halldorsson, B. V, Zusmanovich, P., … Stefansson, K. (2008). Many sequence variants affecting diversity of adult human height. *Nature Genetics*, *40*(5), 609–15.

Gutierrez, A. P., Lubieniecki, K. P., Davidson, E. A., Lien, S., Kent, M. P., Fukui, S., … Davidson, W. S. (2012). Genetic mapping of quantitative trait loci (QTL) for body-weight in Atlantic salmon (*Salmo salar*) using a 6.5K SNP array. *Aquaculture*, *358–359*, 61–70.

Gutierrez, A. P., Yáñez, J. M., Fukui, S., Swift, B., & Davidson, W. S. (2015). Genome-wide association study (GWAS) for growth rate and age at sexual maturation in Atlantic salmon (*Salmo salar*). *PloS One*, *10*(3).

Hamor, T., & Garside, E. T. (1976). Developmental rates of embryos of Atlantic salmon, *Salmo salar* L., in response to various levels of temperature, dissolved oxygen, and water exchange. *Canadian Journal of Zoology*, *54*(11), 1912–1917.

Hansen, A. J. (1985). Effect of anoxia on ion distribution in the brain. *Physiological Reviews*, *65*(1), 101–48.

He, W., Cao, Z., & Fu, S. (2015). Effect of temperature on hypoxia tolerance and its underlying biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities. *Comparative Biochemistry and Physiology , Part A 187*, 232–241.

Heller, M., Edelstein, P., & Mayer, M. (1975). Membrane-bound enzymes. III. Protease activity in leucocytes in relation to erythrocyte membranes. *Biochimica et Biophysica Acta*, *413*(3), 472–82.

Henderson, C. R. (1975). Best linear unbiased estimation and prediction under a selection model. *Biometrics*, 423–447.

Hermans, M. M., Wisselaar, H. A., Kroos, M. A., Oostra, B. A., & Reuser, A. J. (1993). Human lysosomal alpha-glucosidase: functional characterization of the glycosylation sites. *The Biochemical Journal*, *289*, 681–6.

Hochachka, P. W., Buck, L. T., Doll, C. J., & Land, S. C. (1996). Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(18), 9493–9498.

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine Freshwater Research*, 50, 839-66.

Holeton, G. F., & Randall, D. J. (1967). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *The Journal of Experimental Biology*, *46*(2), 317–327.

Hori, K., Sen, A., & Artavanis-Tsakonas, S. (2013). Notch signaling at a glance. *Journal of Cell Science*, *126*(10), 2135–2140.

Houghton, J., Ding, Y., Griggs, D. J., Noguer, M., der Linden, P. J. Van, Dai, X., … Johnson, C. A. (2001). IPCC 2001: Climate Change 2001. *The Climate Change Contribution of Working Group I to the Third Assessment Report of the Intergovemmental Panel on Climate Change*, *159*.

Inoue, H. (1972). Water exchange in a net cage stocked with fish, hamachi. *Bulletin of the Japanese Society of Scientific Fisheries*, *38*(2), 167-176.

Jha, A. R., Zhou, D., Brown, C. D., Kreitman, M., Haddad, G. G., & White, K. P. (2016). Shared genetic signals of hypoxia adaptation in drosophila and in high-altitude human populations. *Molecular Biology and Evolution*, *33*(2), 501–517.

Jibb, L. A., & Richards, J. G. (2008). AMP-activated protein kinase activity during metabolic rate depression in the hypoxic goldfish , *Carassius auratus*, 3111–3122.

Johansen, K.A., & Overturf, K. (2006). Alterations in expression of genes associated with muscle metabolism and growth during nuritional restriction and refeeding in rainbow trout. *Comparative Biochemistry and Physiology, Part B 144*, 119-127.

Johansson, D., Juell, J.E., Oppedal, F., Stiansen, J.E., & Ruohonen, K. (2007). The influence of the pycnocline and cage resistance on current flow, oxygen flux and swimming behaviour of Atlantic salmon (*Salmo salar* L.) in production cages. *Aquaculture*, *265*(1), 271–287.

Johansson, D., Ruohonen, K., Kiessling, A., Oppedal, F., Stiansen, J.E., Kelly, M., & Juell, J.E. (2006). Effect of environmental factors on swimming depth preferences of Atlantic salmon (*Salmo salar* L.) and temporal and spatial variations in oxygen levels in sea cages at a fjord site. *Aquaculture*, *254*(1–4), 594–605.

Joyce, W., Ozolina, K., Mauduit, F., Ollivier, H., Claireaux, G., & Shiels, H. A. (2016). Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. *Biology Letters*, *12*(1), 20150708.

Kaper, J. M. (1976). Molecular organization and stabilizing forces of simple RNA viruses. V. The role of lysyl residues in the stabilization of cucumber mosaic virus strain S. *Virology*, *71*(1), 185–98.

Katso, R., Okkenhaug, K., Ahmadi, K., Timms, J., & Waterfield, M. D. (2001). Cellular function of phisphoinositide 3-kinases: implications for development, immunity, homeostasis, and cancer. *Annual Review of Cell and Developmental Biology*, *17*, 615-675.

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods*, *9*, 29.

Kramer, D. L., & McClure, M. (1982). Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. *Environmental Biology of Fishes*, *7*(1), 47–55.

Lappano, R., Rigiracciolo, D., De Marco, P., Avino, S., Cappello, A.R., Rosano, C., … De Francesco, E.M. (2016). Recent advances on the role of G protein-coupled receptors in hypoxia-mediated signaling. *AAPS Journal*, *18* (2), 305-310.

Landry, C. A., Steele, S. L., Manning, S., & Cheek, A. O. (2007). Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*(2), 317–323.

Lettre, G., Jackson, A. U., Gieger, C., Schumacher, F. R., Berndt, S. I., Sanna, S., … Hirschhorn, J. N. (2008). Identification of ten loci associated with height highlights new biological pathways in human growth. *Online*, *40*(5), 584–591.

Lewis, J. M., Costa, I., Val, A. L., Almeida-Val, V. M., Gamperl, A. K., & Driedzic, W. R. (2007). Responses to hypoxia and recovery: repayment of oxygen debt is not associated with compensatory protein synthesis in the Amazonian cichlid, *Astronotus ocellatus*. *The Journal of Experimental Biology*, *210*(Pt 11), 1935–1943.

Liang, W., Ouyang, S., Shaw, N., Joachimiak, A., Zhang, R., & Liu, Z.-J. (2011). Conversion of D-ribulose 5-phosphate to D-xylulose 5-phosphate: new insights from structural and biochemical studies on human RPE. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *25*(2), 497–504.

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Matthew, P., Leong, J. S., … Vik, J. O. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature*, *533*(6020).

Lillis, A. P., Duyn, L. B. Van, Murphy-ullrich, J. E., & Dudley, K. (2009). LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies. *Physiological Reviews*, *88*(3), 887–918.

Lord, C.C., Thomas, G., & Brown, J.M. (2013). Mammalian alpha beta hydrolase domain (ABDH) proteins: lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. *Biochim Biophys Acta, 1831* (4), 792-802.

López, M. E., Neira, R., & Yáñez, J. M. (2014). Applications in the search for genomic selection signatures in fish. *Frontiers in Genetics*, *5*, 1–12.

Lowe, T. E., & Wells, R. M. G. (1996). Primary and secondary stress responses to line capture in the blue mao mao. *Journal of Fish Biology*, *49*(2), 287–300.

Lutz, P. L., Nilsson, G. E., & Prentice, H. M. (2003). The brain without oxygen : causes of failure--physiological and molecular mechanisms for survival. *Kluwer Academic Pub*.

Marine Harvest. (2016). Salmon Farming Industry Handbook 2016 Marine Harvest. *Salmon Farming Industry Handbook 2016 Marine Harvest*, 93.

McBryan, T. L., Healy, T. M., Haakons, K. L., & Schulte, P. M. (2016). Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *Journal of Experimental Biology*, *219*(4), 474–484.

McCormick, S.D., Regish, A.M., Christensen, A.K., & Björnsson, B.T. (2013). Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. *The Journal of Experimental Biology*, *216*, 1142-1151.

McKinney, G. J., Waples, R. K., Seeb, L. W., & Seeb, J. E. (2016). Paralogs are revealed by proportion of heterozygotes and deviations in read ratios in genotyping by sequencing data from natural populations. *Molecular Ecology Resources*, 1–14.

Moen, T., Hayes, B., Baranski, M., Berg, P. R., Kjøglum, S., Koop, B. F., … Lien, S. (2008). A linkage map of the Atlantic salmon (*Salmo salar*) based on EST-derived SNP markers. *BMC Genomics*, *9*, 223.

Mohlke, K. L., Boehnke, M., & Abecasis, G. R. (2008). Metabolic and cardiovascular traits: An abundance of recently identified common genetic variants. *Human Molecular Genetics*, *17*(R2), 102–108.

Motyka, R., Norin, T., Petersen, L.H., Huggett, D.B., & Gamperl, A.K. (2016). Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss*), but does not affect their upper thermal tolerance. Manuscript submitted for publication.

Msangi, S., Kobayashi, M., Batka, M., Vannuccini, S., Dey, M. M., & Anderson, J. L. (2013). Fish to 2030: prospects for fisheries and aquaculture. *World Bank Report*, 83177–GLB.

Nagy, A., Csanyi, V., Bakos, J., & Horvath, L. (1980). Development of a short-term laboratory system for the evaluation of carp growth in ponds. *Bamidgeh*, *32*(1), 6–15.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., … Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, *405*, 1017–1024.

Nikinmaa, M. (2002). Oxygen-dependent cellular functions—why fishes and their aquatic environment are a prime choice of study. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *133*(1), 1–16.

Nikinmaa, M., & Huestis, W. . (1984). Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. *Journal of Experimental Biology*, *113*(1), 215–224.

Nilsson, G. . (1996). Brain and body oxygen requirements of *Gnathonemus petersii*, a fish with an exceptionally large brain. *The Journal of Experimental Biology*, *199*(Pt 3), 603–7.

Nilsson, G. ., & Östlund-Nilsson, S. (2008). Does size matter for hypoxia tolerance in fish ? *Biology Reviews*, *83*, 173–189.

Nilsson, G. E. (2007). Gill remodeling in fish--a new fashion or an ancient secret? *The Journal of Experimental Biology*, *210*(Pt 14), 2403–2409.

Nilsson, G. E., Östlund-nilsson, S., & Munday, P. L. (2010). Effects of elevated temperature on coral reef fishes : Loss of hypoxia tolerance and inability to acclimate. *Comparative Biochemistry and Physiology, Part A*, *156*(4), 389–393.

Niswender, C.M., & Conn, P.J. (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual Review of Pharmacology and Toxicology, *50*, 295-322.

Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, *41*(1), 199–219.

Noma, A. (1983). ATP-regulated K+ channels in cardiac muscle. *Nature*, *305*, 147-148.

Nordborg, M., & Tavaré, S. (2002). Linkage disequilibrium: What history has to tell us. *Trends in Genetics*, *18*(2), 83–90.

Olowo, J. P., & Chapman, L. J. (1996). Papyrus swamps and variation in the respiratory behaviour of the African fish *Barbus neumayeri*. *African Journal of Ecology*, *34*(2), 211–222.

Oppedal, F., Dempster, T., & Stien, L. H. (2011). Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. *Aquaculture*, *311*(1–4), 1–18.

Parakati, R., & DiMario, J.X. (2002). Sp1- and Sp3-mediated transcriptional regulation of the fibroblast growth factor receptor 1 gene in chicken skeletal muscle cells. *The Journal of Biological Chemistry*, *277*, 9278-9285.

Pedersen, C. L. (1987). Energy budgets for juvenile rainbow trout at various oxygen concentrations. *Aquaculture*, *62*(3–4), 289–298.

Pédron, N., Artigaud, S., Infante, J.-L. Z., Le Bayon, N., Charrier, G., Pichereau, V., & Laroche, J. (2017). Proteomic responses of European flounder to temperature and hypoxia as interacting stressors: Differential sensitivities of populations. *Science of The Total Environment*, *586*, 890–899.

Perry, S. F. (2011). Respiratory Responses to Hypoxia in Fishes. Encyclopedia of Fish Physiology: From Genome to Environment. *Fish Physiology*, *3*, 1751-1756.

Perry, S. F., Jonz, M. G., & Gilmour, K. M. (2009). Oxygen sensing and the hypoxic ventilatory response. *Fish Physiology*, *27*, 193-253.

Pichavant, K., Person-Le-Ruyet, J., Bayon, N. Le, Severe, A., Roux, A. Le, Boeuf, G., … Boeuf, G. (2001). Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. *Journal of Fish Biology*, *59*, 875–883.

Poli, A., Beraudi, A., Villani, L., Storto, M., Battaglia, G., Gerevini, V.D.G., Cappuccio, I., Caricasole, A., D'Onofrio, M., & Nicoletti, F. (2003). Group II metabotropic glutamate receptors regulate the vulnerability to hypoxic brain damage. The Journal of Neuroscience, *23* (14), 6023-6029.

Price, A., Zaitlen, N., Reich, D., & Patterson, N. (2010). New approaches to population stratification in genome-wide association studies. *Nature Reviews Genetics*, *11*(7), 459–463.

Rabalais, N. N., Turner, R. E., Dortch, Q., Justic, D., Jr, V. J. B., & Jr, W. J. W. (2002). Nutrient-enhanced productivity in the northern Gulf of Mexico: past, present and future. *Hydrobiologia*, *475*(1), 39–63.

Randall, D. J., & Ferry, S. F. (1992). 4 Catecholamines. *Fish Physiology*, *12*, 255–300.

Remen, M., Oppedal, F., Imsland, A. K., Olsen, R. E., & Torgersen, T. (2013). Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation. *Aquaculture*, *416–417*, 41–47.

Remen, M., Oppedal, F., Torgersen, T., Imsland, A. K., & Olsen, R. E. (2012). Effects of cyclic environmental hypoxia on physiology and feed intake of post-smolt Atlantic salmon: initial responses and acclimation. *Aquaculture*, *326*, 148–155.

Ren, D., Navarro, B., Perez, G., Jackson, A.C., Hsu, S., Shi, Q., Tilly, J.L., & Clapham, D.E. (2001). A sperm ion channel required for sperm motility and male fertility. Nature, *413*, 603-609.

Richards, J. (2009). Metabolic and molecular responses of fish to hypoxia. *Encyclopedia of Fish*, *27*, 443-485.

Richards, J. . (2011). Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *The Journal of Experimental Biology*, *214*(Pt 2), 191–199.

Richards, J. G., Wang, Y. S., Brauner, C. J., Gonzalez, R. J., Patrick, M. L., Schulte, P. M., … Val, A. L. (2007). Metabolic and ionoregulatory responses of the Amazonian cichlid, *Astronotus ocellatus*, to severe hypoxia. *Journal of Comparative Physiology B*, *177*(3), 361–374.

Rogers, N. J., Urbina, M. A., Reardon, E. E., Mckenzie, D. J., & Wilson, R. W. (2016). Themed Issue Article : Conservation Physiology of Marine Fishes A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (P crit). *Conservation Physiology*, *4*, 1–19.

Rombough, P. J. (1988). Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Canadian Journal of Zoology*, 651–660.

Schmidtko, S., Stramma, L., & Visbeck, M. (2017). Decline in global oceanic oxygen content during the past five decades. *Nature*, *542*(7641), 335–339.

Scott, M. A., Dhillon, R. S., Schulte, P. M., & Richards, J. G. (2014). Physiology and performance of wild and domestic strains of diploid and triploid rainbow trout (*Oncorhynchus mykiss*) in response to environmental challenges. *Canadian Journal of Fisheries and Aquatic Sciences*, *72*(1), 125–134.

Seafish. (2012). Responsible Sourcing Guide: farmed Atlantic salmon.

Shen, C., Nettleton, D., Jiang, M., Kim, S. K., & Powell-Coffman, J. A. (2005). Roles of the HIF-1 hypoxia-inducible factor during hypoxia response in *Caenorhabditis elegans*. *Journal of Biological Chemistry*, *280*(21), 20580–20588.

Slatkin, M. (2008). Linkage disequilibrium--understanding the evolutionary past and mapping the medical future. *Nature Reviews. Genetics*, *9*(6), 477–85.

Smith, W., Houlihan, F., Brechin, J. G., & Houlihan, F. (1996). Tissue-specific changes in protein in crucian carp synthesis rates in vivo during anoxia in crucian carp. *The American Journal of Physiology*, *271*, (4 Pt 2): R897-904.

Sodeland, M., Gaarder, M., Moen, T., Thomassen, M., Kjøglum, S., Kent, M., & Lien, S. (2013). Genome-wide association testing reveals quantitative trait loci for fillet texture and fat content in Atlantic salmon. *Aquaculture*, *408–409*, 169–174.

Sun, H., & Feng, Z. (2013). Neuroprotective role of ATP-sensitive potassium channels in cerebral ischemia. *Acta Pharmacologica Sinica*, *34*(1), 24–32.

Teame, T., Natarajan, P., Zebib, H., Abay, G., T, T., P, N., … G, A. (2016). Report of fish mass mortality from Lake Hashenge, Tigray, Northern Ethiopia and investigation of the possible causes of this event. *International Journal of Fisheries and Aquaculture*, *8*(2), 14–19.

Tian, C., Plenge, R. M., Ransom, M., Lee, A., Villoslada, P., Selmi, C., … Seldin, M. F. (2008). Analysis and application of European genetic substructure using 300 K SNP information. *PLoS Genetics*, *4*(1), 0029–0039.

Timmerman, C. M., & Chapman, L. J. (2004). Hypoxia and interdemic variation in *Poecilia latipinna*. *Journal of Fish Biology*, *65*(3), 635–650.

Tinker, A., Aziz, Q., & Thomas, A. (2014). The role of ATP-sensitive potassium channels in cellular function and protection in the cardiovascular system. *British Journal of Pharmacology*, *171*(1), 12–23.

Tocher, D.R., Bell, J.G., Dick, J.R., Henderson, R.J., McGhee, F., Michell, D., & Morris, P.C. (2000). Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. *Fish Physiology and Biochemistry*, *23*, 59-73.

Tondera, D., Santel, A., Schwarzer, R., Dames, S., Giese, K., & Klippel, A. (2004). Knockdown of mtp18 , a novel phosphatidylinositol 3-kinase-dependent protein , affects mitochondrial morphology and induces apoptosis *. *The Journal of Biological Chemistry*, *279*(30), 31544–31555.

Tosh, J.J., Ventura, R.V., Ang, K.P., Elliott, J.A.K., Kent, M.P., Lien, S., Boulding, E.G., & Schaeffer, L.R. (2014). Genome-wide association analysis of harvest weight in a north American Atlantic salmon population. Proceedings, 10th World Congress on Genetics Applied to Livestock Production.

Tsai, H., Hamilton, A., Guy, D.R., Tinch, A.E., Bishop, S., & Houston, R.D. (2015). Verification of SNPs associated with growth traits in two populations of farmed Atlantic salmon. *International Journal of Molecular Sciences*, *17*, 5.

Tsai, H., Hamilton, A, Tinch, A.E., Guy, D.R., Gharbi, K., Stear, M.J., Matika, O., Bishop, S.C., & Houston, R.D. (2015). Genome wide association and genomic prediction for growth traits in juvnile farmed Atlantic salmon using a high density SNP array. *BMC Genomics*, *16*, 969.

van Ginneken, V. J. T., Onderwater, M., Olivar, O. L., & van den Thillart, G. E. (2001). Metabolic depression and investigation of glucose/ethanol conversion in the European eel (*Anguilla anguilla* Linnaeus 1758) during anaerobiosis. *Thermochimica Acta*, *373*(1), 23–30.

vanRaaij, M. T. M., Pit, D. S. S., Balm, P. H. M., Steffens, A. B., vandenThillart, G. E. E. J. M., van Raaij, M. T. M., … van den Thillart, G. E. (1996). Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. *Hormones and Behavior*, *30*(1), 85–92.

Vaquer-Sunyer, R., & Duarte, C. M. (2011). Temperature effects on oxygen thresholds for hypoxia in marine benthic organisms. *Global Change Biology*, *17*(5), 1788–1797.

Vianen, G. J., Thillart, G. Van Den, Kampen, M. Van, Heel, T. I. Van, & Steffens, A. B. (2001). Plasma lactate and stress hormones in common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) during stepwise decreasing oxygen levels. *Netherlands Journal of Zoology*, *51*(1), 33–50.

Vigen, J. (2008). Oxygen variation within a seacage. *European Master in Aquaculture and Fisheries*. Department of Biology, Univeristy of Bergen.

Virani, N.A., & Rees, B.B. (2000). Oxygen consumption, blood lactate and inter-individual variation in the gulf killifish, *Fundulus grandis*, during hypoxia and recovery. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *126*, 397-405.

Vries, L. De, Zheng, B., Fischer, T., Elenko, E., & Farquhar, M. G. (2000). The regulator of G protein signaling family. *Annual Review of Pharmacology and Toxicology*, *40*, 2235-71.

Wang, X., Liu, S., Dunham, R., & Liu, Z. (2017). Effects of strain and body weight on low-oxygen tolerance of channel catfish (*Ictalurus punctatus*). *Aquaculture International*. DOI 10.1007/s10499-017-0125-2.

Wang, Y., Nakayama, M., Pitulescu, M. E., Schmidt, T. S., Bochenek, M. L., Sakakibara, A., … Adams, R. H. (2010). Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature*, *465*(7297), 483–486.

Wang, Lefevre, S., Thanh Huong, D. T., Cong, N. van, & Bayley, M. (2009). The effects of hypoxia on growth and digestion. *Fish Physiology*, *27*(8), 361–396.

Wang, & Richards, J. G. (2011). Anaerobic Metabolism in Fish. *Encyclopedia of Fish*, 3, 1757-1763.

Waversveld, J. Van, Addink, A. D. F., & van den Thillart, G. (1989). The anaerobic energy metabolism of goldfish determined by simultaneous direct and indirect calorimetry during anoxia and hypoxia. *Journal of Comparative Physiology B*, *159*(3), 263–268.

Weedon, M. N., Lango, H., Lindgren, C. M., Wallace, C., Evans, D. M., Mangino, M., … Frayling, T. M. (2008). Genome-wide association analysis identifies 20 loci that influence adult height. *Nature Genetics*, *40*(5), 575–583.

Weiss, R. F. (1970). The solubility of nitrogen, oxygen and argon in water and seawater. *Deep-Sea Research*, *17*, 721–735.

Welker, T. L., McNulty, S. T., & Klesius, P. H. (2007). Effect of sublethal hypoxia on the immune response and susceptibility of channel catfish, *Ictalurus punctatus*, to enteric septicemia. *Journal of the World Aquaculture Society*, *38*(1), 12–23.

Wells, R. M. G. (2009). Blood-gas transport and hemoglobin function: adaptations for functional and environmental hypoxia. *Fish Physiology*, *27*, 255-299.

Wells, R. M. G., Grigg, G. C., Beard, L. A., & Summers, G. (1989). Hypoxic responses in a fish from a stable environment: blood oxygen transport in the Antarctic fish *Pagothenia borchgrevinki*. *Journal of Experimental Biology*, *141*(1), 97–111.

Wieser, W., & Krumschnabel, G. (2001). Hierarchies of ATP-consuming processes: direct compared with indirect measurements, and comparative aspects. *The Biochemical Journal*, *355*(Pt 2), 389–395.

Wildish, D. J., Keizer, P. D., Wilson, A. J., & Martin, J. L. (1993). Seasonal changes of dissolved oxygen and plant nutrients in seawater near salmonid net pens in the macrotidal Bay of Fundy. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*(2), 303–311.

Withler, R. E., Supernault, J., Swift, B., Peterson, R., & Fukui, S. (2007). Microsatellite DNA assignment of progeny to parents enables communal freshwater rearing in an Atlantic salmon selective breeding program. *Aquaculture*, *272*(1), S318.

Withler, R. E., Supernault, K. J., & Miller, K. M. (2005). Genetic variation within and among domesticated Atlantic salmon broodstocks in British Columbia, Canada. *Animal Genetics*, *36*(1), 43–50.

Wood, S. C., & Johansen, K. (1973). Blood oxygen transport and acid-base balance in eels during hypoxia. *The American Journal of Physiology*, *225*(4), 849–851.

Wood, T. (1986). Physiological functions of the pentose phosphate pathway. *Cell Biochemistry & Function*, *4*, 241-247.

Wu, R. S. S. (1982). Periodic defaunation and recovery in a subtropical epibenthic community, in relation to organic pollution. *Journal of Experimental Marine Biology and Ecology*, *64*(3), 253–269.

Wu, R. S. S. (2009). Effects of hypoxia on fish reproduction and development. *Fish Physiology*, *27*, 79–141.

Xu, J., Tong, H., Wang, L., Hurt, C. M., & Pelleg, A. (1993). Endogenous adenosine, A1 adenosine receptor, and pertussis toxin sensitive guanine nucleotide binding protein mediate hypoxia induced AV nodal conduction block in guinea pig heart in vivo. *Cardiovascular Research*, *27*, 134–140.

Yasuda, S., Kai, M., Imai, S., Takeishi, K., Taketomi, A., Toyota, M., Kanoh, H., & Sakane, F. (2009). Diacylglycerol kinase η augments C-Raf activity and B-Raf/C-Raf heterodimerization. *Journal of Biological Chemistry*, *284*, 29559-29570.

Zhao, K., Aranzana, M. J., Kim, S., Lister, C., Shindo, C., Tang, C., … Nordborg, M. (2007). An Arabidopsis example of association mapping in structured samples. *PLoS Genetics*, *3*(1), 0071–0082.

Ziello, J. E., Jovin, I. S., & Huang, Y. (2007). Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale Journal of Biology and Medicine*, *80*(2), 51–60.

Appendix A

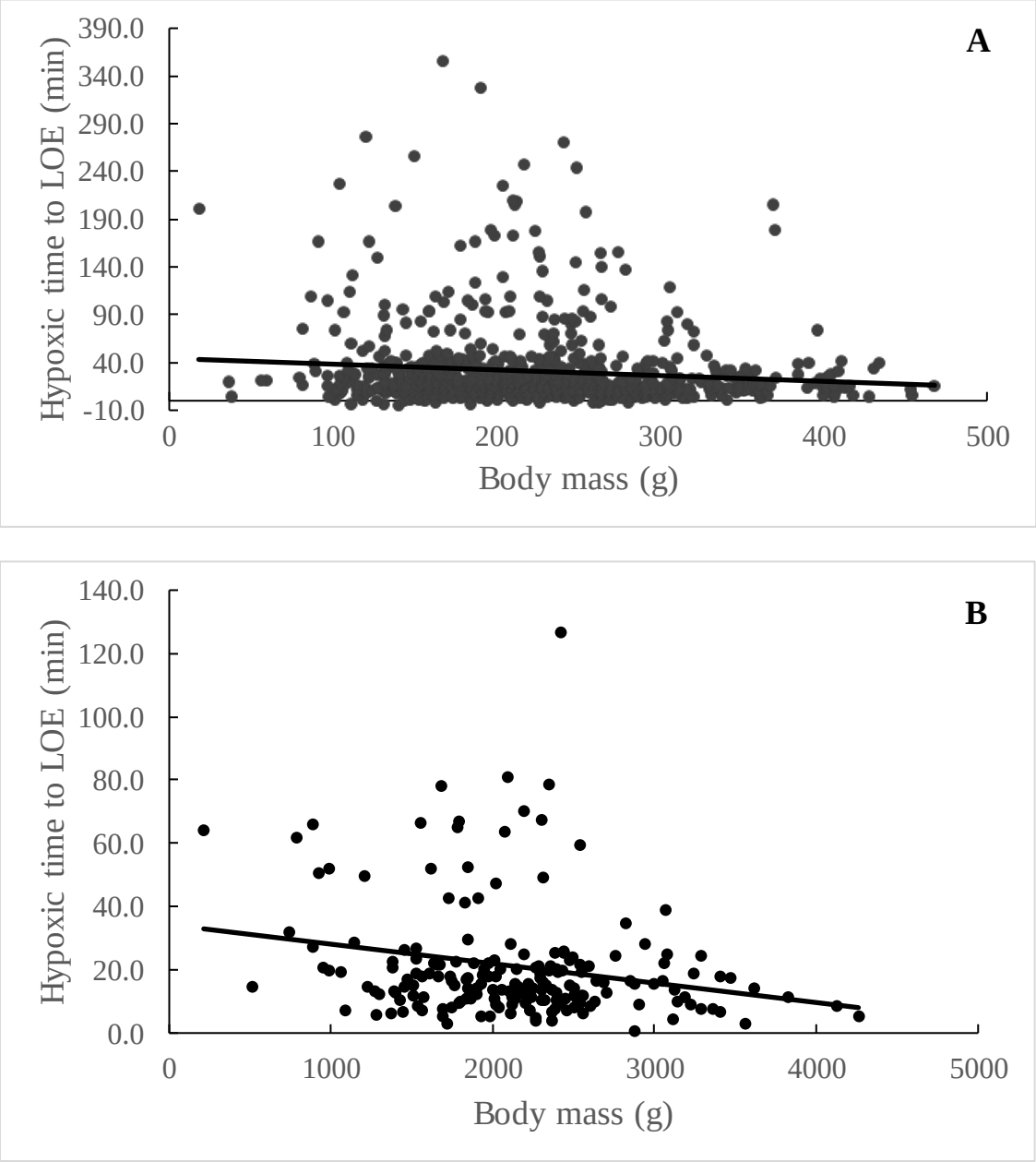

Figure A.1. (A) The correction between hypoxia tolerance and body mass of 855 Atlantic salmon smolts ($\boldsymbol{y = -0.0598x + 44.263}$, $r^2 = 0.011$) and (B) the correction between hypoxia tolerance and body mass of 198 Atlantic salmon adults ($\boldsymbol{y = -0.0062x + 34.242}$, r^2=0.051).