THE IMPACTS OF ELEVATED TEMPERATURE AND MODERATE HYPOXIA ON THE PRODUCTION CHARACTERISTICS, CARDIAC MORPHOLOGY AND HAEMATOLOGY OF ATLANTIC SALMON (*Salmo salar*)

By

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A Thesis submitted
to the School of Graduate Studies in partial fulfillment of the
requirements for the degree of Master of Science Aquaculture

Graduate Program in Aquaculture
Memorial University of Newfoundland

June 2019

St. John's, Newfoundland and Labrador

Abstract

Salmon in sea cages are exposed to high water temperatures and low dissolved oxygen levels (i.e., hypoxia) that are predicted to worsen with climate change. I examined the impacts of an incremental temperature increase (1^{o}C/week to 20^{o}C or 23^{o}C) and moderate hypoxia (~70% air saturation) on the production characteristics, cardiac morphometrics and haematology of farmed Atlantic salmon (*Salmo salar*) of Saint John River origin under laboratory conditions. While temperatures up to 20^{o}C had few negative effects on the salmon, hypoxia significantly reduced feed consumption and growth, and further temperature increases adversely affected the fish. Specific growth rate and feed consumption dropped dramatically after 20^{o}C, and mortalities began at 21^{o}C and reached ~ 30% by 23^{o}C. Salmon exposed to high temperature alone had increased relative ventricular mass (by 21%), whereas those exposed to high temperature and hypoxia had a 15% greater blood oxygen carrying capacity. This research suggests that further increases in coastal ocean temperatures may negatively affect farmed salmon production, especially if combined with hypoxia.

Acknowledgements

My gratitude goes to my supervisor, Dr. A. Kurt Gamperl, who gave me valuable instructions, helpful information and contributed immensely to the overall success of my thesis. To my supervisory committee members: Dr. Matthew Rise, Cyr Couturier and Dr. Dounia Hamoutene for their advice and words of encouragement, I say 'thank you'.

I would like to acknowledge the assistance of Gamperl lab members in conducting this research: Dr. Fabio Zanuzzo who designed and organized the experiments, performed the haematological measurements and guided me through the process of analyzing and graphing the data; Rebecca Sandrelli who performed the cardiac morphometric measurements; Dr. Anne Beemelmanns who introduced me to the R statistical package and helped to develop an appropriate statistical model; Gord Nash, Zoe Zrini, MacGregor Parent, Ellen Peroni and Tasha Harrold who assisted with the collection of samples and care of the fish in the Laboratory for Atlantic Salmon and Climate Change Research. In addition, I would like to thank Mr. Danny Boyce and the staff of the Dr. Joe Brown Aquatic Research Building (JBARB) for fish care / husbandry prior to the beginning of experiments. This research was conducted as part of the 'Mitigating the Impact of Climate-Related Challenges on Salmon Aquaculture' project, and funded by the Atlantic Canada Opportunities Agencies, Innovate NL and Innovation PEI.

I cannot forget to say I cherish you my wife, Olabisi, for your great support, love and understanding, together with our adorable, inestimable and precious children, Oluwatamilore and Moyinoluwa. God bless you.

Above all, I thank God the 'Alpha and Omega' who granted me wisdom, knowledge and understanding to complete this M.Sc. program.

Dedication

This thesis is dedicated to the glory and honour of the Almighty God, without whom I am nothing.

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List of Symbols, Abbreviations and Nomenclature

Abbreviation	Definition
%	Percent
^{o}C	Degree Celsius
[ ]	Chemical concentration
~	Approximate
<	Less than
±	Plus or minus
#	Number
µl	Microliter
air sat.	Air saturation
ANOVA	Analysis of variance
CT_{max}	Critical thermal maximum
DFO	Department of Fisheries and Oceans
DFA	Department of Fisheries and Aquaculture
DO	Dissolved oxygen
FC	Feed consumption
FCR	Feed conversion ratio
g	Gram
Hb	Haemoglobin
Hct	Haematocrit
h	Hour
HSI	Hepato-somatic index

JBARB	Joe Brown Aquatic Research Building
LASCCR	Laboratory for Atlantic Salmon and Climate Change Research
LOS	Limiting oxygen saturation
K	Condition Factor
m^3	Cubic metre
ml	Milliliter
mM	Millimoles per litre
mm	Millimeter
N	Sample size
N_2	Nitrogen
NL	Newfoundland
O_2	Oxygen
OCLTT	Oxygen and capacity limitation of thermal tolerance
PCA	Principle Component Analysis
PIT	Passive integrated transponder
PO_2	Partial pressure of oxygen
ppt	Parts per thousand
RVM	Relative ventricular mass
S.E.	Standard error of the mean
SGR	Specific growth rate
SSI	Spleen-somatic index
TMS	Tricaine methane-sulphonate
vs	Versus

1.0. Background

Aquaculture is currently the world's primary source of fish and shellfish products. Further, the decline in wild fisheries, human population growth and an increase in global per capita fish consumption (Turchini et al., 2009; FAO, 2016a; Parker, 2018) will require an increase in aquaculture production by 40% to meet the projected demand for fish (186 Million tonnes by 2030; World Bank, 2012). The Atlantic salmon (*Salmo salar*) industry is one of the world's most economically important aquaculture sectors (Bostock et al., 2010), with an annual production of 2 million tonnes worth $14.6 billion, and is ranked eighth in total value globally amongst cultured fish species (FAO, 2016b). The top salmon producing countries in 2016 were Norway, Chile, Scotland, Canada, Australia and the Faroe Islands (Marine Harvest, 2015). The Atlantic salmon is the main species produced by the Canadian aquaculture industry, and represents 70% and 80% of its total production and value, respectively (DFO, 2017a). British Columbia is Canada's largest producer of Atlantic salmon (85,608 tonnes in 2017), while New Brunswick produces the most salmon on Canada's east coast (23,867 tonnes in 2017) (DFO, 2017b). That said, both Nova Scotia and Newfoundland (NL) are expanding production. A blueprint for the development of the NL salmon industry was initiated in 2006, a year in which production was 73,000 tonnes and the industry's total market value was $52.3 Million (DFA, 2006). Between 2006 and 2015 the value of the NL salmon industry increased by 169.6% (to $149 million, DFA, 2015) and production was approx. 25,400 tonnes in 2016 (approx. $263 million). Further expansion of the Atlantic salmon industry in NL is also anticipated as current producers (Cooke Aquaculture and Mowi Canada East) look to expand, and Grieg Seafoods NL is planning to set up operations in the province and to

produce up to 35,000 tonnes per annum using cage sites in Placentia Bay. Thus, total production of Atlantic salmon in NL is anticipated to exceed 50,000 tonnes by 2022 (DFA, 2017).

1.1. Environmental Conditions at Sea Cages

The production of Atlantic salmon using marine sea cages (net-pens) located close to the coastline is increasing worldwide (Lekang et al., 2016), and salmon restricted sea cages are exposed to a range of environmental conditions that cannot be controlled (Johansson et al., 2006; Oppedal et al., 2011a; Burt et al., 2012; Stehfest et al., 2017; Solstorm et al., 2018). These changes in environmental conditions (i.e., salinity, water currents, light, high waves / winds, water temperature, oxygen levels and chemical treatments used during production) may have a negative impact on salmon health / welfare and production (Oppedal et al., 2011a; Remen et al., 2013; Hevrøy et al., 2013, 2015; Vikeså et al., 2017). Salmon are known to be highly temperature sensitive, and oxygen is considered to be a limiting factor for fish metabolism and production (Remen et al., 2012a, 2012b, 2013, 2016; Vikeså et al., 2016, 2017; Oldham et al., 2019). However, the impacts of temperature extremes and low dissolved oxygen levels (hypoxia) on farmed Atlantic salmon within aquaculture sea cages need to be better understood so that the industry can better cope with, and adapt to, these conditions and climate change.

1.2. Temperature

Given that salmon (like all fishes) are sensitive to changes in temperature, fluctuations in this parameter that occur within sea cages can be a significant challenge

(Olsvik et al., 2013; Stehfest et al., 2017). For example, winter water temperatures can be a problem for sea caged Atlantic salmon as 'super chill' can occur when temperatures drop below the freezing point of salmon blood (-0.7^{o}C). Under these conditions the salmon's blood may crystallize and the heart can stop beating, resulting in high mortalities (Saunders, 1991; Boghen, 1995). For example, the 'super chill' event at NL cage-sites during the winter months of 2014 led to large losses (up to 73.1% of the 2013 production value) (DFA, 2015). 'Super chill' is a major challenge to the salmon aquaculture industry, most especially in the coastal regions of Nova Scotia where frequent low temperatures associated with high tides in late February and early March occur every five to seven years (CBC, 2015). Interestingly, climate change might have benefits in this regard, as winter temperatures may not decrease to such low levels in the future.

The industry is also concerned about high temperatures that occur during the summer / fall. Sea surface temperatures have been higher during the past three decades compared to any other time dating back to 1880 (Finnis, 2013), and climate change is predicted to increase ocean temperatures further (by 1.5-2^{o}C) by the end of the 21st century (Meehl et al., 2007; IPCC, 2018). Temperatures within sea cages can increase by approximately 1^{o}C per week from early spring to late summer / early fall when they reach their maximum values. However, these maximum surface water temperatures vary among locations: maximum values of 18^{o}C have been experienced at salmon cage-sites on the south coast of NL during the summer / fall months; 20^{o}C has been reported at cage-sites in Fortune Bay, Newfoundland and Norway; while maximum values as high as 23^{o}C have

been experienced by Tasmanian salmon populations at cage-sites (Johansson et al., 2006; Mansour et al., 2008; Oppedal et al., 2011b; Burt et al., 2012; Stehfast et al., 2017).

Salmon are highly temperature sensitive and have the capacity to detect and avoid temperatures that are beyond their optimal range by vertically distributing themselves within the sea cages (Jobling, 1981; Oppedal et al., 2011a; Stehfast et al., 2017). It has been reported that salmon within sea cages prefer water temperatures that are optimal for their growth and survival (16-18^{o}C), but avoid high temperatures beyond 18^{o}C (Johansson et al., 2006). These data are consistent with Jobling (1981) who suggested that salmon growth was optimal at 14-18^{o}C, and Stehfest et al. (2017) who reported a thermal preference range of 16.5-17.5^{o}C for Tasmanian Atlantic salmon within sea cages. However, identifying optimal temperatures for salmon confined within sea cages may be difficult due to constant changes in water conditions (Johansson et al., 2009). For example, reduced growth and feed conversion have been reported in salmon exposed to temperatures of 16^{o}C (Oppedal et al., 2011a; Remen et al., 2012a) and 17^{o}C (Vikeså et al., 2017).

Surprisingly, there is a paucity of data on the impacts of long-term incremental temperature increases (as seen within sea cages in many locations throughout the world) on production parameters, and the physiology and health / welfare of cultured salmon. Although data from short-term laboratory experiments (i.e., CT_{max}) provide information about maximum temperatures that salmon can be exposed to before they are negatively impacted (Barnes et al., 2011; Anttila et al., 2013), there are a number of confounding factors that make the application of these CT_{max} data to the sea cage environment questionable. Reported CT_{max} values could overestimate the thermal tolerance of salmon

in sea cage conditions. First, the upper temperature that a fish can tolerate is dependent on the rate of temperature increase (the faster the temperature increase, the higher the CT_{max}; Lutterschmidt and Hutchison, 1997; Galbreath et al., 2004; Mora and Maya, 2006; Madeira et al., 2012; Vinagre et al., 2015; Moyano et al., 2017), and although high CT_{max} values in excess of 25^{o}C have been reported for Atlantic salmon when exposed to temperature increases of 0.3^{o}C min^{-1} to 2^{o}C h^{-1} (Elliott, 1991; Elliott and Elliott, 1995; Anttila et al., 2013; Penney et al., 2014; Anttila et al., 2015; Bowden et al., 2018), there is no literature that establishes the upper thermal tolerance of farmed Atlantic salmon exposed to incremental temperature increases (approximately 1^{o}C per week) that simulate sea cage conditions. Second, salmon can alter their biology / physiology when exposed to changes in temperature for extended periods (weeks) and this allows them to adjust to the new temperature, and potentially to tolerate higher temperatures. For example, salmon may be able to tolerate high temperatures as a result of acclimation or genetic adaptation (Remen et al., 2013), and Anttila et al. (2015) recently showed that long-term acclimation increases the Atlantic salmon's thermal CT_{max} by 2.2^{o}C. Finally, different populations of fish (i.e., fish of different genetic background) may have quite different upper thermal tolerances as demonstrated in many fish species (Meffe et al., 1995; Doyle, et al., 2011). Anttila et al. (2013) showed that there were significant differences in upper temperature tolerance (CT_{max}) and hypoxia tolerance among 41 families of juvenile, cultured, Atlantic salmon of Saint John River origin. In contrast, however, Anttila et al. (2015) reported that wild Atlantic salmon from southern and northern Norway responded very similarly when challenged with increases in temperature, and suggested that physiological plasticity might allow salmon populations to compensate for warmer future ocean temperatures.

Ultimately, understanding the impacts of incremental increases in sea cage water temperatures on salmon physiology and biology will help to predict the salmon's response to climate change, and to determine the maximum temperature that various populations / stocks can tolerate before their welfare and production are compromised.

1.3. Hypoxia

The incidence and severity of hypoxia (a decline in water oxygen levels) have increased in recent decades, and are predicted to worsen with increasing global temperatures (Levin and Breitburg, 2015; IPCC, 2018). Hypoxic conditions can occur in sea cages due to oxygen consumption by the fishes (Diaz and Breitburg, 2009), increased water temperatures (which reduce water oxygen levels by 10 to 20% for every 10^{o}C increase in water temperature; Farrell and Richards, 2009), lower rates of water exchange, and other factors that limit oxygen availability within the cages (Johansson et al., 2006, 2007; Oppedal et al. 2011b; Burt et al., 2012). These hypoxic events have led to massive fish deaths worldwide (Azanza et al., 2005; Bouchet et al., 2007; Vaquer-Sunyer and Duarte, 2008; Blucher, 2015). Severe hypoxic events leading to the loss of ~ 85,000 farmed adult Atlantic salmon (valued at ~ $1 million) have been reported in Tasmania (Blucher, 2015), and large-scale losses of milkfish (*Chanos chanos*) in Bolinao, the northern Philippines, resulted in an economic loss of about $120,000 to this industry (Azanza et al., 2005). Recently, on the South Coast of NL, a site stocked with 882,000 Atlantic salmon experienced considerable fish mortality in 2012 due to a decrease in dissolved oxygen in the cages, and this resulted in the remaining fish being either harvested or moved to another site (Hamoutene et al., 2018).

Seasonal variations in dissolved oxygen (DO) levels, with high oxygen levels (~ 100% air sat.) in the spring and low oxygen concentrations (~ 50 - 70% air sat.) in the autumn are frequently experienced in Atlantic salmon sea cages (Crampton et al., 2003; Johansson et al., 2006, 2007; Burt et al., 2012). For example, it has been shown that salmon produced in Norway experience drops in water oxygen levels (i.e., to 60% air sat. or less) which may cause chronic stress and have a negative impact on salmon production, physiology and welfare (Johansson et al., 2007). It has been reported that Tasmanian salmon avoid hypoxic waters (< 35% air sat.) at the bottom of their sea cages, but also that they spend a great deal of time in waters with sub-optimal DO levels (< 60% air sat.) during late summer / early autumn to avoid warm surface temperatures (Stehfest et al., 2017). Finally, hypoxic events are a major concern to the salmon aquaculture industry along the British Columbia (Canada) coast as frequent changes in ocean currents during the summer result in the upwelling of large pockets of deep water that are low in oxygen (Marine Harvest, 2015). Studies have shown that Atlantic salmon exposed to hypoxic conditions below a threshold of 70% air saturation have reduced growth and feed consumption (Remen et al., 2012a, b; Remen et al., 2016; Vikeså et al., 2017). However, there are limited available data (e.g., Burt et al., 2012) on how long-term exposure to moderate hypoxic conditions (i.e., at 70% air sat.) impacts the physiology, immunology and health / welfare of Atlantic salmon produced in Atlantic Canada.

The capacity of salmon reared in Tasmania to detect and avoid severe hypoxic conditions (< 35% air sat.) near the bottom of their sea cages, and survive hypoxic events via vertical habitat contraction, suggests that these salmon have a higher hypoxia tolerance than reported earlier (Stehfest et al., 2017). This may be due to either

acclimation or genetic adaptation (Stehfest et al., 2017). However, earlier studies conducted on post-smolt Atlantic salmon subjected to cyclic hypoxia (40, 50, 60 or 70% air saturation) at 16^{o}C revealed that acclimation had no effect on hypoxia tolerance (Remen et al., 2012a). Further, survival of Atlantic salmon during hypoxic events (~60 – 70% air sat.) observed at sea cages on the south coast of Newfoundland in the summer months indicates that these fish are tolerant to these water oxygen levels (Burt et al., 2012).

1.4. Combined Effects of Temperature and Hypoxia

High temperatures, in combination with hypoxia, are one of the most challenging environmental conditions facing fish in aquaculture, and there are a number of studies that have looked at the combined effects of these stressors on fishes (Mendonça and Gamperl, 2010; Anttila et al., 2013; McBryan et al., 2013; Motyka et al., 2017; Vikeså et al., 2016). However, there have only been a few studies that have looked at the effects of these two environmental challenges on Atlantic salmon production characteristics. Vikeså et al. (2016) reported that both moderate hypoxic conditions (70% air sat.) and low dietary energy intake significantly ($P < 0.05$) reduced growth and feed consumption, and resulted in higher mortality, in post-smolt Atlantic salmon held at 17^{o}C. Atlantic salmon exposed to a temperature of 16^{o}C under hypoxic conditions of 70, 60, 50 and 40% air saturation had reduced feed consumption by 13, 15, 63 and 73%, respectively, compared to the Control group at 90% air saturation, while their final weights were 17 and 11% lower at 40 and 50% air saturation (Remen et al., 2012a). Finally, reduced appetite was observed in salmon exposed to 16^{o}C when DO dropped to 70% air saturation, while

growth and feed conversion were significantly reduced ($P < 0.05$) at 50% air saturation (Oppedal et al., 2011a). These studies show that even water temperatures that are considered optimal for Atlantic salmon (16-18^{o}C), when combined with moderate hypoxia, can have a significant negative impact on salmon growth and survival (Oppedal et al., 2011a; Remen et al., 2012a; Vikeså et al., 2016). Therefore, it is important to understand the combined impact of these two environmental conditions, as experienced by salmon in sea cages, on their physiology, health / welfare and production characteristics.

While it is certain that these two environmental parameters impact Atlantic salmon, it is unclear how they will interact as they vary under marine culture conditions. For example, their effects could be synergistic, antagonistic, additive or multiplicative (McBryan et al., 2013). The well accepted, but also controversial (e.g., see Jutfelt et al., 2018), concept of "oxygen and capacity limitation of thermal tolerance" (OCLTT; Pörtner and Knust, 2007; Pörtner, 2010; Pörtner et al., 2017), suggests that a mismatch between oxygen demand and supply (i.e., a reduction in aerobic scope) under conditions of high temperature and low oxygen levels would result in negative impacts on fishes and other aquatic organisms. This idea is consistent with a number of studies which show that hypoxia reduces thermal tolerance and that high temperatures reduce hypoxia tolerance (Caposella et al., 2012; McBryan et al., 2013; Verberk et al., 2013; Lapointe et al., 2014). However, Anttila et al. (2015) showed that the acclimation of Atlantic salmon to 15^{o}C (vs. 5^{o}C) and diel cycling hypoxia (from normoxia to 63-67% air saturation) had both antagonistic and synergistic effects on the capacity of these fish to tolerate these abiotic stressors. For example, the thermal tolerance (CT_{max}) of warm acclimated salmon

increased with warm acclimation by 2.2°C, but decreased (by 0.4°C) when warm temperature was combined with overnight hypoxia. In contrast, the combination of these stressors more than doubled hypoxia tolerance. This study shows that salmon (fishes) can have unexpected species-specific effects which will influence their survival capacity and performance when exposed to combined stressors.

1.5. Determinants of Thermal / Hypoxia Tolerance

The OCLTT hypothesis suggests that oxygen delivery to the tissues is the primary factor determining a fish's metabolic capacity and aerobic scope, and thus, their thermal and hypoxia tolerance (Farrell, 2002; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Anttila et al., 2013; Motyka et al., 2017; Muñoz et al., 2018). Therefore, it would be expected that tolerance to these two environmental challenges would be associated with the heart's pumping capacity and/or the oxygen carrying capacity of the blood (i.e., arterial oxygen convection). Indeed, Anttila et al. (2013) showed that the relative ventricular mass of 41 families of Atlantic salmon was correlated significantly with their acute thermal tolerance (CT_{max}), and that CT_{max} and hypoxia tolerance were also correlated, and Muñoz et al. (2018) showed that haematocrit was positively correlated with CT_{max} in Chinook salmon (*Oncorhynchus tshawytscha*). However, it is difficult to predict whether, and to what extent, heart morphometrics and / or blood oxygen carrying capacity (haematocrit and blood haemoglobin levels) would be affected / impacted by an incremental temperature increase with or without hypoxia. This is because experimentally-induced anemia failed to affect the CT_{max} of European perch (*Perca fluvitalis*; Brijs et al., 2015) or sea bass (*Dicentrachus labrax*; Wang et al., 2014), and

while hypoxic-acclimation has been found to increase the haematocrit and blood haemoglobin levels of some fish species (Wu, 2002; Timmerman and Chapman, 2004; Lai et al., 2006; Borowiec et al., 2015; Martos-Sitcha et al., 2017), and Motyka et al. (2017) and Harter et al. (2019) did not find that these two parameters were impacted by chronic hypoxia (~ 40% air saturation) in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon held at 10^{o}C, respectively. While acclimation to higher temperatures increases the CT_{max} of Atlantic salmon, relative ventricle mass is lower in fish acclimated to warmer temperatures (Klaiman et al., 2011; Anttila et al., 2015; Keen et al., 2017). Further, cardiac performance is associated with aspects of cardiac morphology other than RVM. For example, the amount of compact myocardium of the ventricle is important for the pressure generating capacity of the heart (Gamperl et al., 1995; Farrell, 2002; Farrell et al., 2009; Klaiman et al., 2011; Anttila et al., 2015; Motyka et al., 2017), and Anttila et al. (2015) showed that the combination of higher temperature (~ 15 vs. 8^{o}C) and diel cycling hypoxia increased the percentage of compact myocardium in the ventricle. Also, although cardiac shape (e.g., ventricular length to width ratio) has been shown to differ between wild vs. hatchery salmonids (Poppe et. al., 2003) and to determine the heart's pumping capacity (Claireaux et al., 2005), to my knowledge, no studies have reported how this parameter is related to the upper thermal tolerance of fishes.

1.6. Thesis Objectives

Given the uncertainty about how climate change might affect / impact the production characteristics and survival of farmed Atlantic salmon of Saint John River origin, I subjected Atlantic salmon from Northern Harvest Sea Farms to: 1) an

incremental temperature increase (12 - 20^{o}C at 1^{o}C / week) and then 20^{o}C for an additional 3 weeks (Experiment $^{\#}$1); and 2) an incremental increase in temperature until mortalities reached ~30% (i.e., to estimate the thermal tolerance of current stocks) (Experiment $^{\#}$2). Further, I conducted these two temperature challenges under both normoxic and hypoxic (~ 70 % air sat.) conditions. An incremental temperature increase of 1^{o}C / week to 20^{o}C was chosen in Experiment $^{\#}$1 to simulate sea cage conditions that may presently occur in sea cages in Atlantic Canada (Burt et al., 2012; Caines, unpubl. data). Seventy percent air saturation was used as the level of hypoxia as decreases in water oxygen levels of this magnitude have been recorded at Atlantic salmon cage-sites in Newfoundland (Burt et al., 2012) and other locations in the North Atlantic (e.g., Crampton et al., 2003; Johansson et al., 2006; 2007) during late summer / early autumn.

In addition, I measured cardiac morphometrics (ventricle mass, RVM, % spongy and compact myocardium; and ventricular shape) and blood haematocrit and haemoglobin levels at the end of Experiment $^{\#}$2 (i.e., in fish exposed to normoxia at 12^{o}C; warmed to 23^{o}C under normoxic conditions; and warmed to 23^{o}C under moderate hypoxia) to examine to what extent these important parameters with respect to cardiac performance and arterial O_2 convection may determine the thermal tolerance of Atlantic salmon.

Co-authorship Statement

The following statements clearly identify my contributions to the development, execution and preparation of this thesis:

1) Design of the Project: Dr. A. Kurt Gamperl and Dr. Fabio Zanuzzo designed the research project, and organized various aspects related to fish care and sampling for Experiments #1 and #2.

2) Practical Aspects of the Research: The author was primarily responsible for the execution (fish care / feeding) of Experiment #1, and participated in the sampling of fishes in both experiments. Data on cardiac morphometrics and haematocrit were collected by Ms Rebecca Sandrelli, while the reported data for haemoglobin were collected by Dr. Fabio Zanuzzo.

3) Data Analysis: Dr. Anne Beemelmanns introduced me to R statistical package and helped to develop an appropriate statistical model. The statistical analyses of the data obtained from all the experiments was conducted by the author using R statistical package, with the exception of analysis of the growth parameters in Experiment #2 which was conducted by Dr. Zanuzzo using the SAS statistical package.

4) Writing of this thesis: This thesis was written in collaboration with my supervisor (Dr. Gamperl).

Authorship for the future publication arising from this research is:

A.K. Gamperl[1], O.O. Ajiboye, F. S. Zanuzzo, R.M. Sandrelli, E. Peroni, A. Beemelmanns. The Impacts of Elevated Temperature and Moderate Hypoxia on the Production Characteristics, Cardiac Morphology and Haematology of Atlantic Salmon (*Salmo salar*).

[1]Corresponding Author

2.0. Materials and Methods

2.1. Experimental Animals

The Atlantic salmon used in these experiments were raised by Northern Harvest Sea Farms in Stephenville (NL) and transferred as smolts to the Dr. Joe Brown Aquatic Research Building (JBARB) located in the Department of Ocean Sciences, Memorial University. The fish were held at the JBARB in 3000 L tanks for 2 to 3 months before experiments began. These tanks were supplied with seawater (32 ppt salinity) maintained at a temperature of 12^{o}C and ~ 100% air saturation, and exposed to a photoperiod of 14 h light: 10 h dark. The fish were fed a daily ration of commercial pellets (EWOS, Cargill Innovation Center, Dirdal, Norway) using belt feeders at ~1% body mass day^{-1}. While being held at the JBARB, the salmon were implanted with Passive Integrated Transponder (PIT) tags (Loligo Systems ISO 11784 certified PIT tags, Denmark), which provide each fish with a unique identification number that can be obtained by non-lethal scanning.

All experimental procedures described herein were approved by Memorial University of Newfoundland's Institutional Animal Care Committee (Protocol # 16-90-KG), and followed guidelines set by the Canadian Council on Animal Care.

2.2. Experiment #1: Impacts of a Prolonged Incremental Temperature Increase to 20°C and Moderate Hypoxia on Atlantic Salmon Production Metrics and Morphometrics

2.2.1. Experimental Protocol

Three hundred and sixty Atlantic salmon with an initial mass of 138.1 ± 1.1 g were randomly distributed into six 2.2 m^3 fiberglass tanks supplied with seawater (32 ppt salinity) and initially maintained at 12°C and 100% air saturation at the Laboratory for Atlantic Salmon and Climate Change Research (LASCCR). Photoperiod was 14 h light: 10 h dark.

After a two week acclimation period, the salmon were randomly assigned to 3 treatments (2 tanks per treatment, 60 fish $tank^{-1}$; stocking density ~ 3.8 kg m^{-3}) as illustrated in Figure 2.1: 1) a temperature of 12°C and 100-110% air saturation for the duration of the experiment (Control group); 2) an incremental temperature increase (1°C / week to 20°C at 100-110% air saturation) and then 20°C for an additional 3 weeks (Warm/Normoxia group); and 3) a decrease in water oxygen content to approximately 70% of air saturation over 1 week, and then the temperature increase as above (Warm/Hypoxia group). These changes / differences in environmental conditions are illustrated in Figure 2.2.

The seawater temperature in four of the six tanks was increased at a rate of 1°C every week (as outlined in Figures 2.1, 2.2A) with the aid of a large plate heat-exchanger that delivered heated seawater to a vacuum degasser that supplied a header tank. Oxygen levels in the header tank were maintained at a level that resulted in ~ 70% air saturation in

the hypoxic tanks (Figure 2.2B, C) and oxygen diffusers were placed in the warm-normoxic tanks to ensure that O_2 levels were above 95% over the course of the experiment.

Seawater temperatures and dissolved oxygen levels in all the experimental tanks were monitored every morning (YSI, ProODO, OH, USA). In addition, a Fibox 3 (LCD V3) meter with pre-calibrated dipping probe (PreSens; Regensburg, Germany), and a computer running LCDPST3 (Version 2.0.1.0) software (PreSens), was used to monitor dissolved oxygen levels in one of the hypoxic tanks continuously. Total ammonia and nitrite levels in the tanks were also monitored (LaMotte test kit, Maryland, USA) on a weekly basis and remained within acceptable levels (i.e., below 0.02 and 0.5 mg L^{-1}, respectively). Fish were fed twice daily to apparent satiation (at 9:00 and 15:00) by hand with commercial salmon feed (EWOS, Cargill Innovation Center, Dirdal, Norway). The fish were fed over a period of ~ 20 minutes (i.e., slowly), and feeding in each tank was stopped when a few pellets started to appear on the bottom of the tank. The pellets at the bottom of the tank were not collected post-feeding, and thus, food consumption and food conversion ratio may be overestimated.

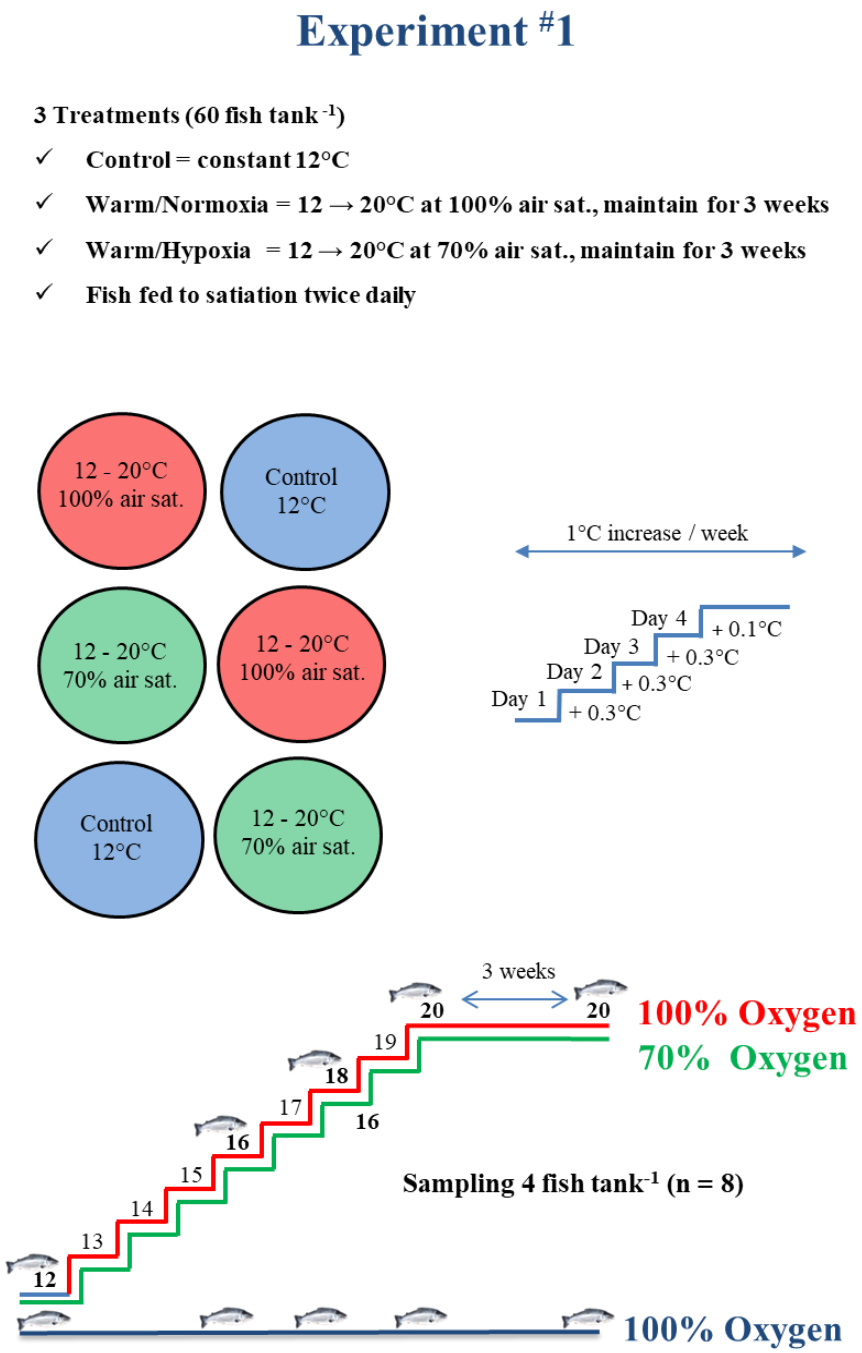

Figure 2.1. Schematic diagram showing the protocol for Experiment #1, where fish were exposed to: a constant temperature of 12°C and normoxia (Control group); or a prolonged incremental temperature increase to 20°C and 3 weeks acclimation at 20°C, with (Warm/Hypoxia) or without (Warm/Normoxia) moderate hypoxia. The salmon were sampled (see fish symbol) at 12, 16, 18 and 20°C (twice) for the measurement of morphometric parameters and the collection of liver tissue for genomic analyses (Beemelmanns et al., in prep). Note: Control fish were sampled at the same time points during the experiment.

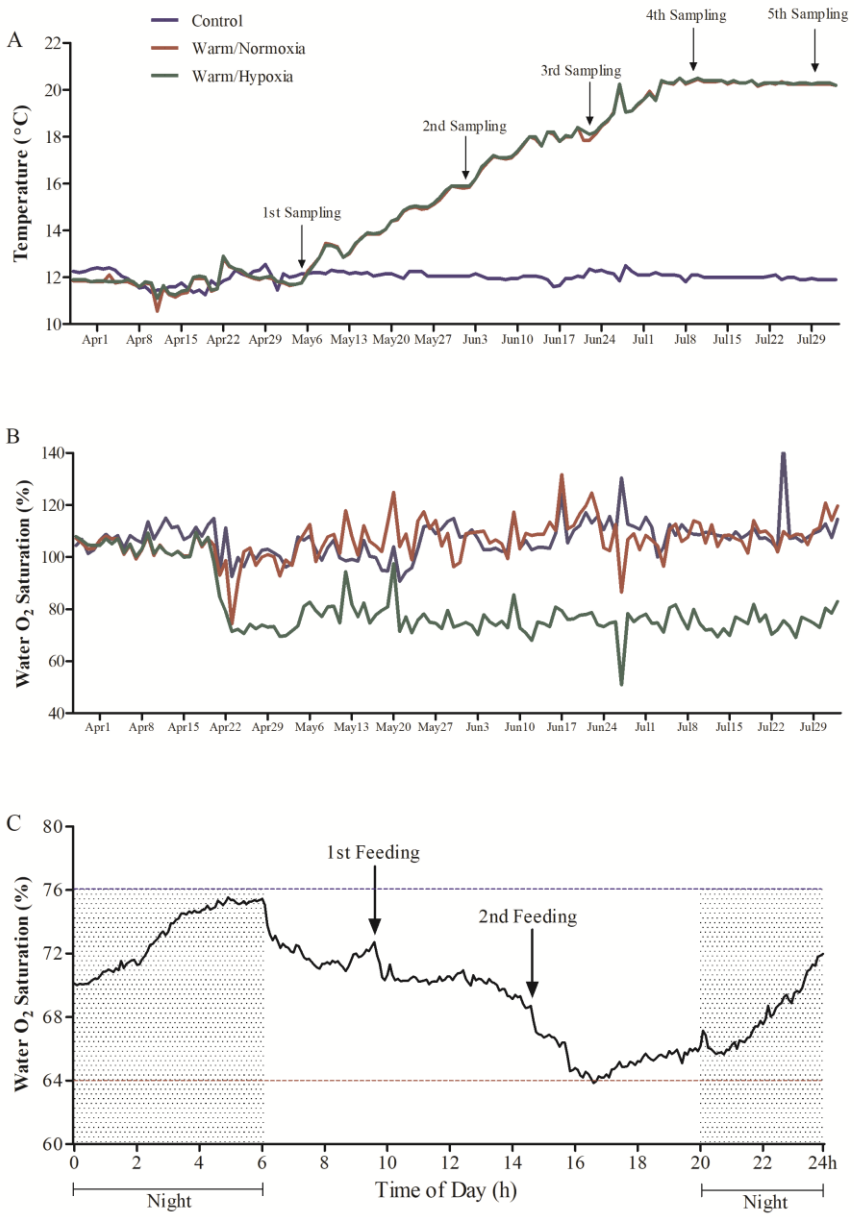

Figure 2.2. Recordings of changes in tank temperature (A) and dissolved oxygen levels (B) for the 3 groups over the course of the experiment. In this experiment, fish were exposed to: a constant temperature of 12°C and normoxia (Control); a prolonged incremental temperature increase to 20°C and 3 weeks acclimation at 20°C (Warm/Normoxia); or the incremental temperature increase combined with moderate hypoxia (~ 70% air saturation; Warm/Hypoxia). (C) Water dissolved oxygen levels (% saturation) in one of the Warm/Hypoxia tanks at 19°C over a period of 24 hours to show how oxygen levels varied over the day, and the effects of feeding.

2.2.2. Growth Morphometrics, Survival and Feed Consumption

Atlantic salmon (8 fish per treatment, 4 per tank) were sampled at 12°C, 16°C, 18°C, 20°C and after being held at 20°C for 3 weeks following euthanasia using 0.4 g L^{-1} TMS-222 (tricaine methane-sulphonate; Syndel Laboratories, BC., Canada). Thereafter, the PIT tag of the fish was read to identify the individual, the mass and fork length of the fish were measured, and the liver, spleen and heart were removed, weighed, and then a piece from each tissue was flash frozen in liquid nitrogen prior to being stored at -80°C. These tissues were used in other analyses conducted by members of the Mitigating the Impacts of Climate-Related Challenges on Salmon Aquaculture (MICCSA) project team.

Specific growth rate (SGR) was calculated according to the formula of Elliott (1975):

$$SGR\ (\text{in \% day}^{-1}) = 100\ [\text{In}\ (W_t - W_{ij})] / t] \qquad (1)$$

where W_t was the weight at sampling (in g); W_i was weight at the beginning of the experiment, and t was growth period in days.

Condition factor (K) was calculated according to the formula of Fulton (1904):

$$K = 100\ [W / (L^3)] \qquad (2)$$

where (W) was the weight (in g) and (L) was the fork length of the fish (in cm).

Feed conversion ratio was estimated as feed consumed per body weight gain, using the feed provided to each tank per day, and dividing it by the number of fish in the tank. This gave us an average value of feed consumed per individual in each tank (i.e. N = 2) per day, and these data were summed over the course of the experiment to provide an estimate of the total amount of food consumed per individual. The hepato-somatic index (HSI) was calculated as 100 [M_L/M_b], where M_L was the liver mass and M_b was body mass (both measured in g); spleen-somatic index (SSI) was calculated as 100 [M_S/M_b] where M_S was the spleen mass (in g); and Relative Ventricular Mass (RVM) was calculated as 100 [M_v/M_b], where M_v was ventricle mass (in g). Mortality is presented as the number of fish that died over the experiment and not at a percentage as fish were sampled at several temperatures in each group (see Figure 2.1).

2.2.3. Statistical Analyses

All data are presented as means ± standard error of the mean (SEM), and were examined to verify normality, independence, and homogeneity of variance before further analyses were performed. Data on growth performance were initially analyzed using a mixed model with sampling and groups as fixed effects and tank as random effects, whereas the final production metrics were analyzed using one-way ANOVAs, with tank as random effects at each sampling point. These analyses were followed by Tukey's post-hoc tests to identify significant differences ($P < 0.05$) between groups at each temperature / time point, or at the end of the experiment. All statistical analyses were performed using the R-statistical package (R Core Team, 2019).

2.3. Experiment #2: Impacts of a Prolonged Incremental Temperature Increase and Moderate Hypoxia on Atlantic Salmon Production Characteristics, Survival, Haematological Parameters and Cardiac Morphometrics

2.3.1. Experimental Protocol

PIT-tagged Atlantic salmon with an initial mass of 494.1 ± 4.4 g were randomly distributed into six 2.2 m^3 fiberglass tanks at the LASCCR. These tanks were supplied with seawater at 12^oC and 100-110% air saturation, with a photoperiod of 14 h light: 10 h dark, for 2 weeks to allow them to acclimate to their holding conditions.

At the end of the two week acclimation period, the Atlantic salmon used in this study were subjected to 3 treatments (52 fish $tank^{-1}$; stocking density ~ 11.5 kg m^{-3}) as described in Figure 2.3.: 1) a constant temperature of 12^oC and 100-110% air saturation for the duration of the experiment (Control group); 2) an incremental increase in temperature (1^oC / week) from 12^oC at 100-110% air saturation until fish mortalities reached ~30% (Warm/Normoxia group); and 3) a decrease in water oxygen content to ~ 70% air saturation over one week, and then an incremental increase in temperature (1^oC / week) from 12^oC until fish mortalities reached ~30% (Warm/Hypoxia group). Temperatures, dissolved oxygen levels and ammonia / nitrite levels were monitored as in Experiment #1. Further, the tanks were monitored several times daily, and any fish that appeared to be moribund (having difficulty maintaining equilibrium; swimming erratically) were immediately removed from the tank and euthanized in seawater containing 0.4 g L^{-1} MS-222. In this experiment, the fish in each tank were hand fed to satiation on the morning of the first two days after a new temperature was reached (i.e., in

the two experimental groups), and the fish were fed 1.5 x this satiation level daily using automatic feeders (from 9:00 AM to 4:00 PM) until the next temperature was reached. Satiation level was then measured again.

2.3.2. Production Characteristics, Survival and Feed Consumption

To monitor production characteristics during the experiment, 10 fish per treatment (5 per tank) were measured at 8 time points (i.e., 12^{o}C, 16^{o}C, 18^{o}C, 19^{o}C, 20^{o}C, 21^{o}C, 22^{o}C and 23^{o}C); 23^{o}C was the temperature where mortalities reached 30% and the experiment was stopped. After being netted from the tank, the fish were briefly anesthetized in 0.1g L^{-1} MS-222, their PIT-tag was read, and the mass and fork length of each fish was measured. These data were used to calculate the fish's SGR and K as above. Feed consumption, HSI and SSI was also calculated as above, and cumulative mortality was calculated based on the number of fish per treatment that became moribund / died at a particular temperature. This value is not expressed in % as fish were sampled from the tanks at several points as temperature increased.

2.3.3. Heart Size and Cardiac Morphology

At the end of the study (i.e., when mortalities in the two experimental groups reached ~30% at 23^{o}C), heart size and cardiac morphometrics were determined in some of the remaining fish given the association between cardiac performance and both hypoxia and upper temperature tolerance (Farrell, 2009; Farrell et al., 2009; Eliason et al., 2011; Anttila et al., 2013, 2015; McBryan et al., 2013). Salmon (n=10 per group; 5 per tank) were netted from the tanks, euthanized in 0.4 g L^{-1} TMS-222 and had their

ventricles removed. The ventricles were rinsed in heparinized phosphate buffered saline (0.85%), blotted dry, weighed, and then fixed in 10% formaldehyde for 72 h. Thereafter, the following morphometric measurements were made on the fixed ventricle. Ventricle length (along the anteroposterior axis) and width (along the left-right axis) were measured as in Poppe et al. (2003). The ventricle was then cut in half, and the compact myocardium of each half of the ventricle was separated ('peeled') from the rest of the ventricle using the tip of a fine spatula and blunt forceps (Farrell et al., 1988). Relative ventricular mass was calculated as in Experiment $^{\#}$1, and the percent compact and spongy myocardium were measured by dividing their total mass by that of the fixed ventricle, and multiplying by 100.

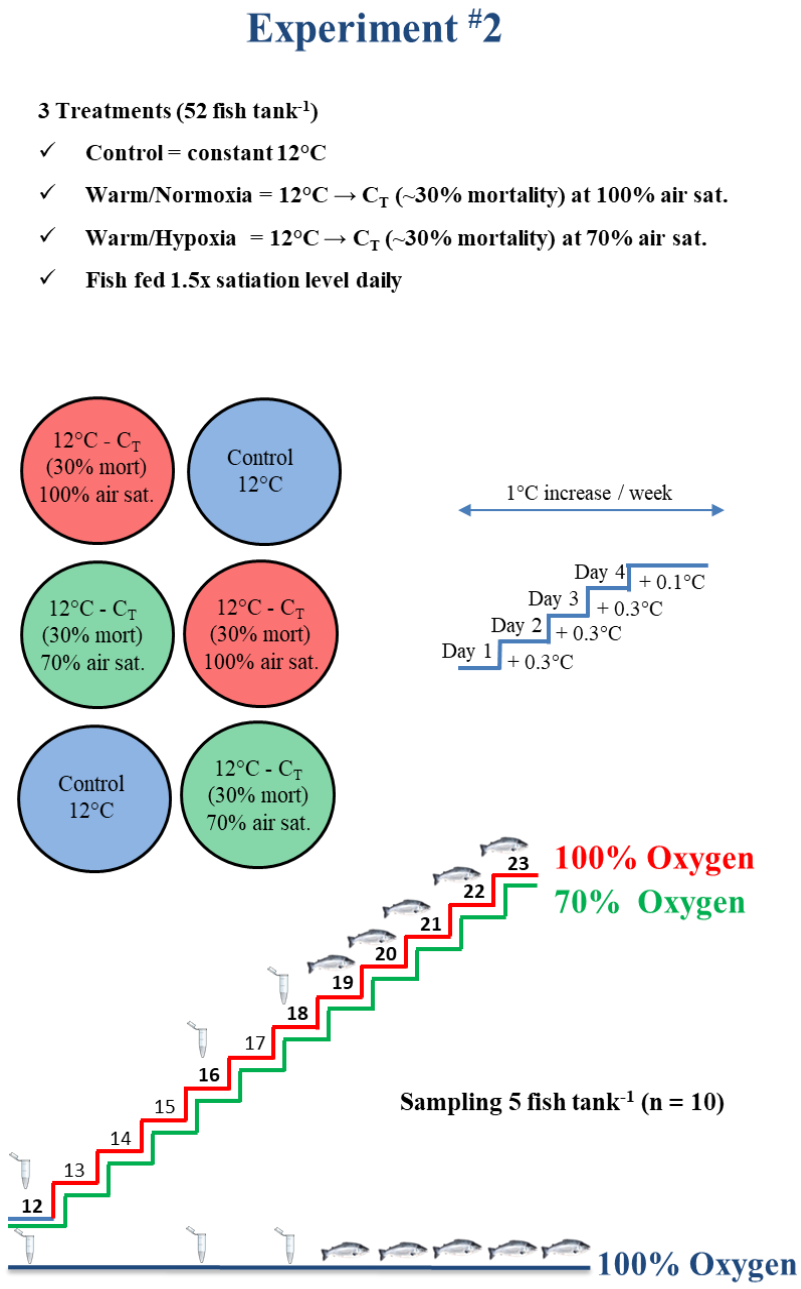

Figure 2.3. Schematic diagram showing the protocol for Experiment #2, where fish were exposed to: a constant temperature of 12°C and normoxia (Control group); or a prolonged incremental temperature increase (1°C per week) with (Warm/Hypoxia) or without hypoxia (Warm/Normoxia) until mortalities reached 30% at 23°C. At lower temperatures a small blood sample was collected from some of the fish, and the fish returned to the tanks (see Eppendorf tubes), whereas at temperatures of 19°C and higher the fish were terminally sampled (see fish symbols). Note: Control fish were sampled at the same time points during the experiment. Fish sampled at temperatures $\leq$ 23°C were used for analysis of blood and tissue parameters indicative of stress (Zanuzzo et al., in prep)

2.3.4. Haematocrit and Haemoglobin

To determine the blood oxygen-carrying capacity of Atlantic salmon subjected to elevated water temperature and moderate hypoxia (70% air saturation), haematocrit (Hct) and blood haemoglobin (Hb) concentration were determined on 10 fish per group at the end of the experiment (i.e., when mortalities in the two experimental groups reached ~30%). After fish had been anaesthetized using 0.4 g L^{-1} MS-222, blood samples (2 ml) were immediately drawn from the caudal vein using 3 ml (heparinized; 100 I.U. mL^{-1}) plastic syringes and 22 gauge needles. A 100 µl subsample of blood was placed into a 2 mL microcentifuge tube for the measurement of blood haemoglobin (Hb) concentration and flash frozen in liquid N_2, and then stored at -80 ^{o}C. Thereafter, two 50 µl capillary tubes were filled with blood, centrifuged for 2 min at 10,000 x g, and the percentage of packed red cells (%) was determined. Haemoglobin concentration was measured using Drabkin's reagent and a SpectraMax M5 microplate reader following manufacturer's instructions (Sigma-Aldrich, Oakville, ON, Canada).

2.3.5. Statistical Analyses

All data are presented as means ± standard error of the mean (SEM), and were examined to verify normality, independence, and homogeneity of variance before further analyses were performed. Data on growth performance were initially analyzed using a mixed model with sampling and groups as fixed effects and tank as a random effect, whereas the cardio-morphometric and hematological parameters, were analyzed using one-way ANOVAs, with tank as a random effect at each sampling point. These analyses were followed by Tukey's post-hoc tests to identify significant differences ($P < 0.05$)

between groups at each temperature / time point, or at the end of the experiment. All statistical analyses were performed using the R-statistical package (R Core Team, 2019).

3.0. Results

3.1. Experiment #1

The first experiment focused on the impacts of an incremental temperature increase (12 to 20^{o}C), then 3 weeks at 20^{o}C, with or without moderate hypoxia (70% air saturation) (see Figure 2.1) on Atlantic salmon production metrics and morphometrics (Figure 2.4, Table 2.1). Fish held at 12^{o}C and normoxia increased in length and weight over the experiment (by ~ 13 cm and 420 g, respectively), and this increase in size appeared to be relatively linear (Figure 2.4A and B). However, SGR (based on the sampling of 8 fish at various time points) revealed a different pattern of fish growth (Figure 2.4E). SGR started out at 1.7% day^{-1}, and then gradually decreased from the 4th week onwards. At the end of the experiment, when the remaining fish were all measured, SGR over the entire experiment was only 1.25% day^{-1} (Table 2.1). This decrease in SGR with time was consistent with the values for feed consumption (FC)(in g fish^{-1} day^{-1}), which plateaued in the middle of the experiment and then decreased slightly even though the fish were increasing in weight (Figure 2.5).

Increasing the temperature to 20^{o}C, and then holding the fish at this temperature for three weeks, had minimal effects on weight, weight gain or SGR as compared to the Control group (Figure 2.4.). This is because the Warm/Normoxia fish ate more food over the experiment (~ 437 vs. 408 g fish^{-1}), but also had an FCR that was ~ 9.7% higher (1.065 vs. 0.971) (Table 2.1). In the Warm/Normoxia group, the relationship between

temperature and FC was bell shaped. It increased from 12^{o}C until 18-19^{o}C (peaking at ~ 20% above that of Control fish), but then fell considerably and was not different from the Control group by the time the fish had been at 20^{o}C for 1 week (Figure 2.5).

Acclimating the fish to ~70% saturation at 12^{o}C did not appear to affect the FC of the fish (Figure 2.5). However, it resulted in a significantly lower weight and weight gain at this temperature (by 13.3 and 29.5% respectively; Figure 2.4A, D). Further, these fish continued to grow slower than the Control and Warm/Normoxia fish over the course of the experiment. For example, weight and weight gain were significantly lower from 13^{o}C onwards (Figure 2.4A, D), and SGR was consistently lower; this difference significant when the fish reached 20^{o}C (Figure 2.4E). At the end of the experiment, this resulted in the fish from the Warm/Hypoxia group being approx. 20% smaller (by weight) and having an SGR that was 15% lower as compared to the other two groups (Table 2.1). This lower growth in the Warm/Hypoxia group as compared to the Control group was related to both lower values for feed consumption (346 g vs. 407 g fish^{-1}, respectively), and a higher FCR (i.e., ~ 1.08 vs. 0.97). Whereas, the difference in growth performance between the Warm/Normoxia and Warm/Hypoxia groups was solely due to a difference in feed consumption (Table 2.1). While changes in FC with increased temperature in the Warm/Hypoxia group reflected those observed for the Warm/Normoxia group, this parameter was consistently much lower (by 26.1%) in the former group (Figure 2.5). These above differences were also reflected in condition factor, which was ~1.03 in the Warm/Hypoxia group at the end of the experiment vs. ~ 1.08 in the Control and Warm/Normoxia groups (Figure 2.4C; Table 2.1). While mortality was higher in the

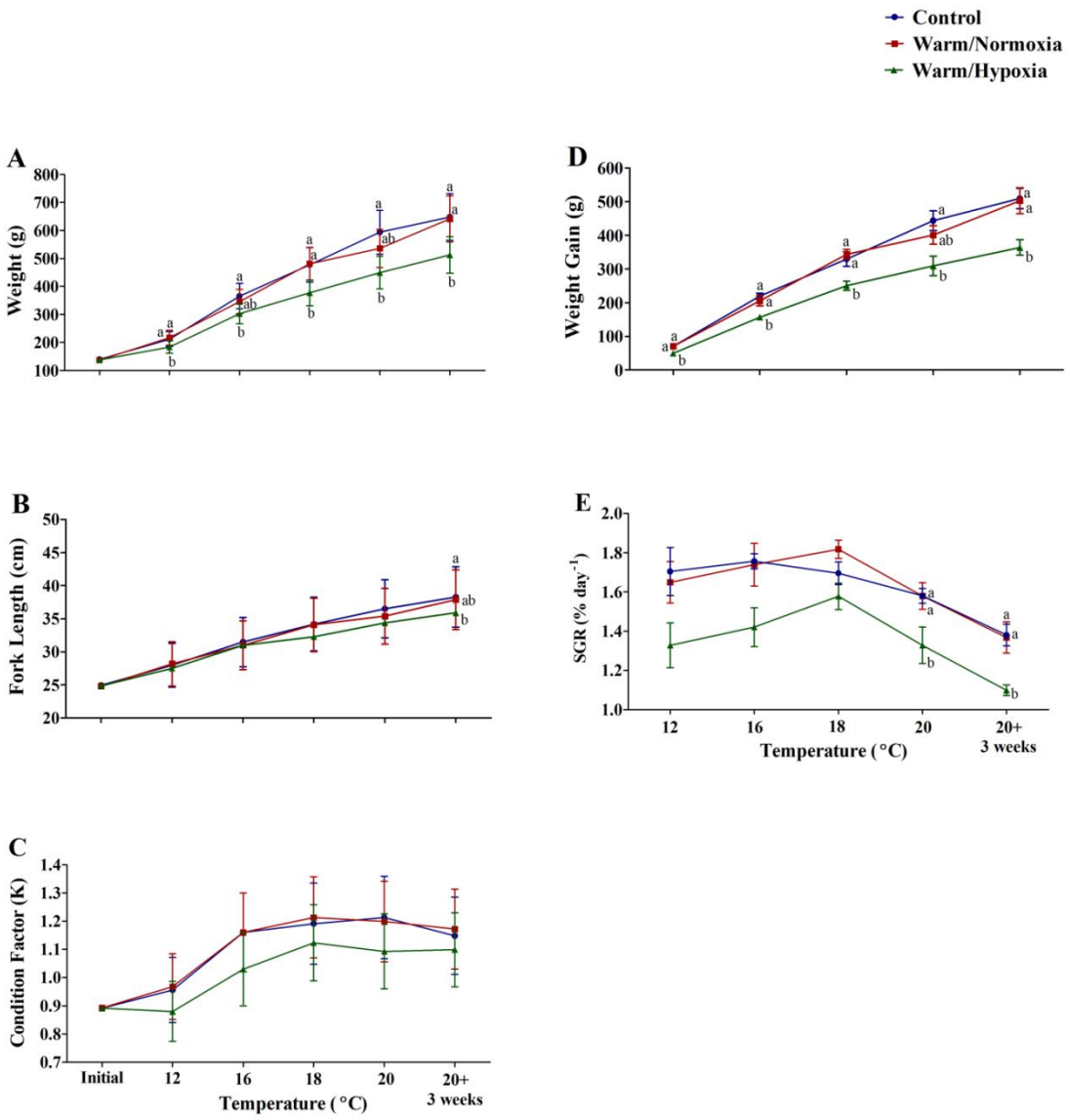

Figure 2.4. The effect of an incremental temperature increase (12 to 20°C), then 3 weeks at 20°C, on Atlantic salmon growth performance when held at 100% air saturation (Warm/Normoxia) or 70% air saturation (Warm/Hypoxia) (N = 8). Control fish were exposed to a constant temperature of 12°C and normoxia. Significant differences between groups at each temperature are indicated by different letters ($P < 0.05$). Weight gain and SGR (panels D and E, respectively) at the temperatures shown were all calculated based on the initial weight of the fish in each group (see panel A). Values are means ± 1 S.E (N = 8).

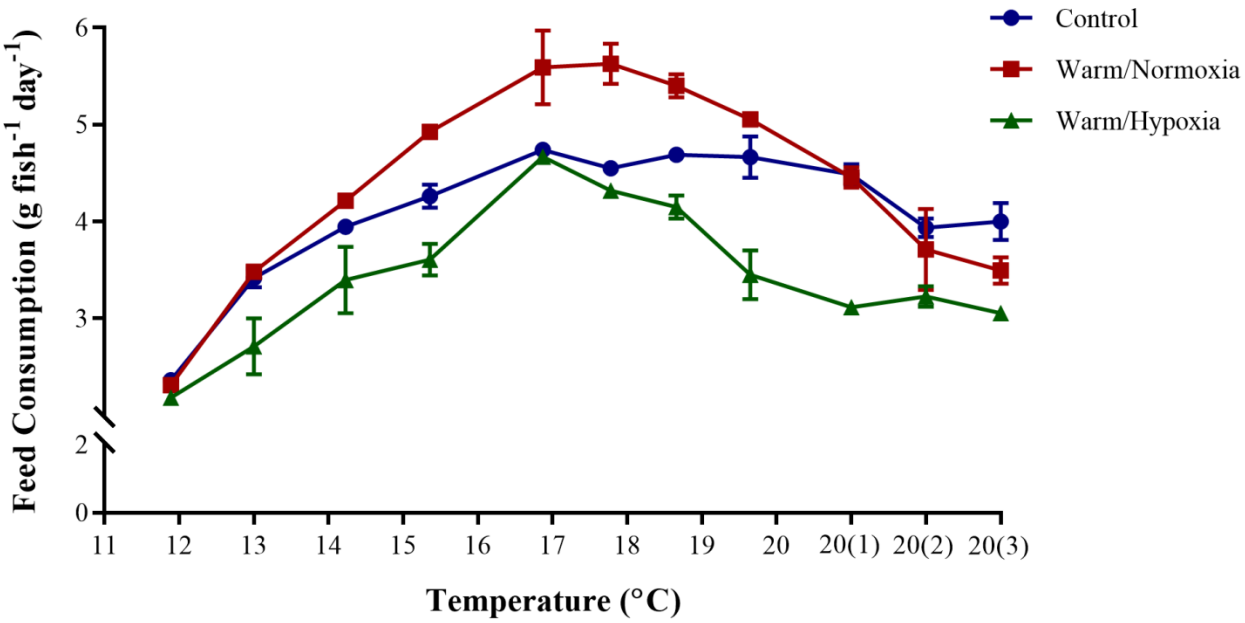

Figure 2.5. The effect of an incremental temperature increase (12 to 20°C), and then 3 weeks at 20°C, on the food consumption of Atlantic salmon held at ~ 100% air saturation (Warm/Normoxia) or ~ 70% air saturation (Warm/Hypoxia). The number in parenthesis following '20' indicates the weeks the fish had been held at this temperature. Control fish were exposed to a constant temperature of 12°C and normoxia. Each value represents the mean of two tanks, the value for each tank the mean of 7 days of feed consumption at that temperature.

Table 2.1. The effect of an incremental temperature increase (12 to 20°C), then 3 weeks at 20°C, on Atlantic salmon production metrics when held at 100% air saturation (Warm/Normoxia) or 70% air saturation (Warm/Hypoxia). Control fish were exposed to a constant temperature of 12°C and normoxia. Different letters indicate significant differences between groups ($P < 0.05$). Values are means ± 1 S.E. N = 77 – 87, except for feed conversion ratio and feed consumption where N = 2 (i.e., 2 tanks per treatment).

	Control	**Warm/Normoxia**	**Warm/Hypoxia**
Initial Weight (g)	136.6 ± 2.3[a]	136.1 ± 2.1[a]	140.3 ± 2.3[a]
Final Weight (g)	556.6 ± 11.3[a]	546.1 ± 12.9[a]	461.0 ± 8.7[b]
Initial Length (cm)	24.8 ± 0.1[a]	24.8 ± 0.1[a]	25.0 ± 0.2[a]
Final Length (cm)	37.8 ± 0.2[a]	36.8 ± 0.3[a]	35.4 ± 0.2[b]
Weight Gain (g)	420.0 ± 10.5[a]	410.0 ± 12.2[a]	320.4 ± 7.8[b]
Condition Factor (K)	1.08 ± 0.01[a]	1.08 ± 0.01[a]	1.03 ± 0.08[b]
Specific Growth Rate (% day^{-1})	1.25 ± 0.02[a]	1.23 ± 0.02[a]	1.06 ± 0.02[b]
Feed Consumption (g $fish^{-1}$)	407.8	436.7	346.2
Feed Conversion Ratio	0.971	1.065	1.082
Cumulative Mortality (fish)	0	2	12[1]

[1]This largely reflects mortality in one tank early during the experiment. The cause of this mortality is unknown.

Warm/Hypoxia group, this reflected an unknown issue in one of the tanks early in the experiment (i.e., at 12^{o}C).

The somatic indices measured were not significantly different between the groups (Figure 2.6 A-C), although RVM in Warm/Hypoxia fish was lower than for Warm/Normoxia fish at P = 0.064. Average values for HSI, SSI and RVM in the three groups were approximately 0.94, 0.081 and 0.073, respectively.

3.2. Experiment $^{\#}$2

The second experiment involved a temperature increase, with or without hypoxia, until mortalities reached approx. 30% in the two experimental groups (see Figure 2.3). Morphometric and growth parameters increased similarly in the three groups between 12 and 19-20^{o}C (Figure 2.7). However, differences between the groups became evident as temperature increased further. For example, weight, fork length and weight gain were lower in the two experimental groups at 20^{o}C (Figure 2.7A, B and D), and weight, fork length, condition factor, weight gain and SGR were approx. 13-23, 2.4-5.7, 10-12, 30-43 and 28-37% lower, respectively, than in the Control group by 23^{o}C (Figure 2.7 A-E). High temperature appeared to have a greater impact on these parameters when combined with hypoxia, but the only difference that reached significance was weight gain at 22^{o}C (Figure 2.7D).

These differences in growth parameters (e.g., weight and SGR) above 20^{o}C were undoubtedly related to the decrease in feed consumption that was associated with increasing temperature (Figure 2.8B). Feed consumption began to fall in the

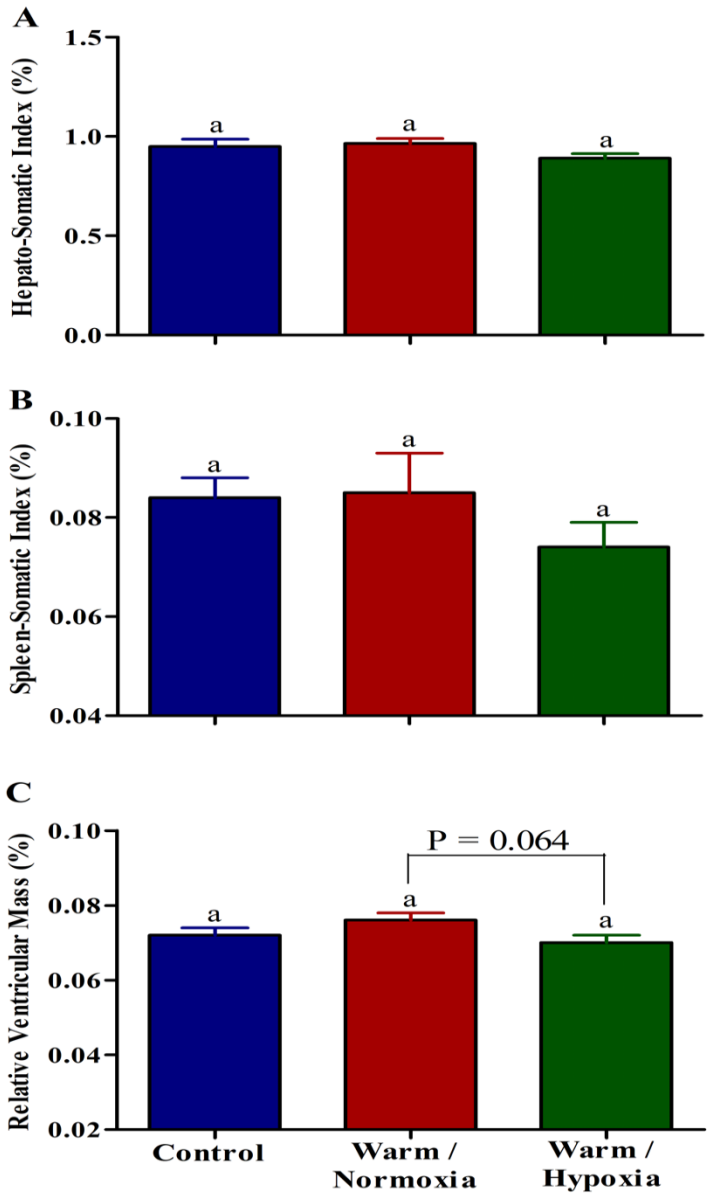

Figure 2.6. The effect of an incremental temperature increase (12 to 20°C), then 3 weeks at 20°C, on the hepato-somatic index (A), spleen-somatic index (B) and relative ventricular mass (C) of Atlantic salmon held at 100% air saturation (Warm/Normoxia) or 70% air saturation (Warm/Hypoxia) (N = 8). Control fish were exposed to a constant temperature of 12°C and normoxia. Significant differences between groups at each temperature are indicated by different letters ($P < 0.05$).

Warm/Normoxia and Warm/Hypoxia groups by 19 and 20^{o}C, respectively, and the fish in these two groups stopped feeding by 21^{o}C and 23^{o}C, respectively. There were a few mortalities at 21^{o}C or below in the two experimental groups (i.e., ~ 2 - 4 fish) (Figure 2.8A). However, mortality increased sharply when temperature was increased to 22^{o}C and then to 23^{o}C in both the Warm/Normoxia and Warm/Hypoxia groups. Mortalities at these temperatures were 6-7 and 11-12 fish, respectively (Figure 2.8A); this corresponded to ~ 15 and 30% cumulative mortality at these temperatures if only the fish that were not sampled during the experiment are considered (64 fish per group).

Surviving fish (10 from each group) were sampled at the end of the experiment (i.e., at 23^{o}C). Exposure to the incremental temperature increase to 23^{o}C (with or without moderate hypoxia) had large effects on both HSI and SSI. HSI was 21 and 27% lower in the Warm/Normoxia and Warm/Hypoxia groups as compared to the Control group, respectively, and SSI was 32 and 40% lower (the former difference not reaching significance, P = 0.106) (Figure 2.9).

While blood oxygen carrying capacity (as measured by Hct and [Hb]) was not significantly different between the Control and Warm/Normoxia groups, the parameters were higher in the Warm/Hypoxia group as compared to the Control group (P = 0.0165 and 0.095, respectively)(Figure 2.10). Ventricle length, width and length / width were approx. 13-15 mm, 9 mm and 1.4-1.6, respectively, and did not differ between three groups (Figure 2.11). There were also no differences in the % compact and % spongy myocardium (~ 35 and 65%, respectively)(Figure 2.12B, C). However, the ventricles of the Warm/Normoxia group were approx. 21% larger than measured in the other two groups (RVM = 0.082 vs. 0.068 %) (Figure 2.12A).

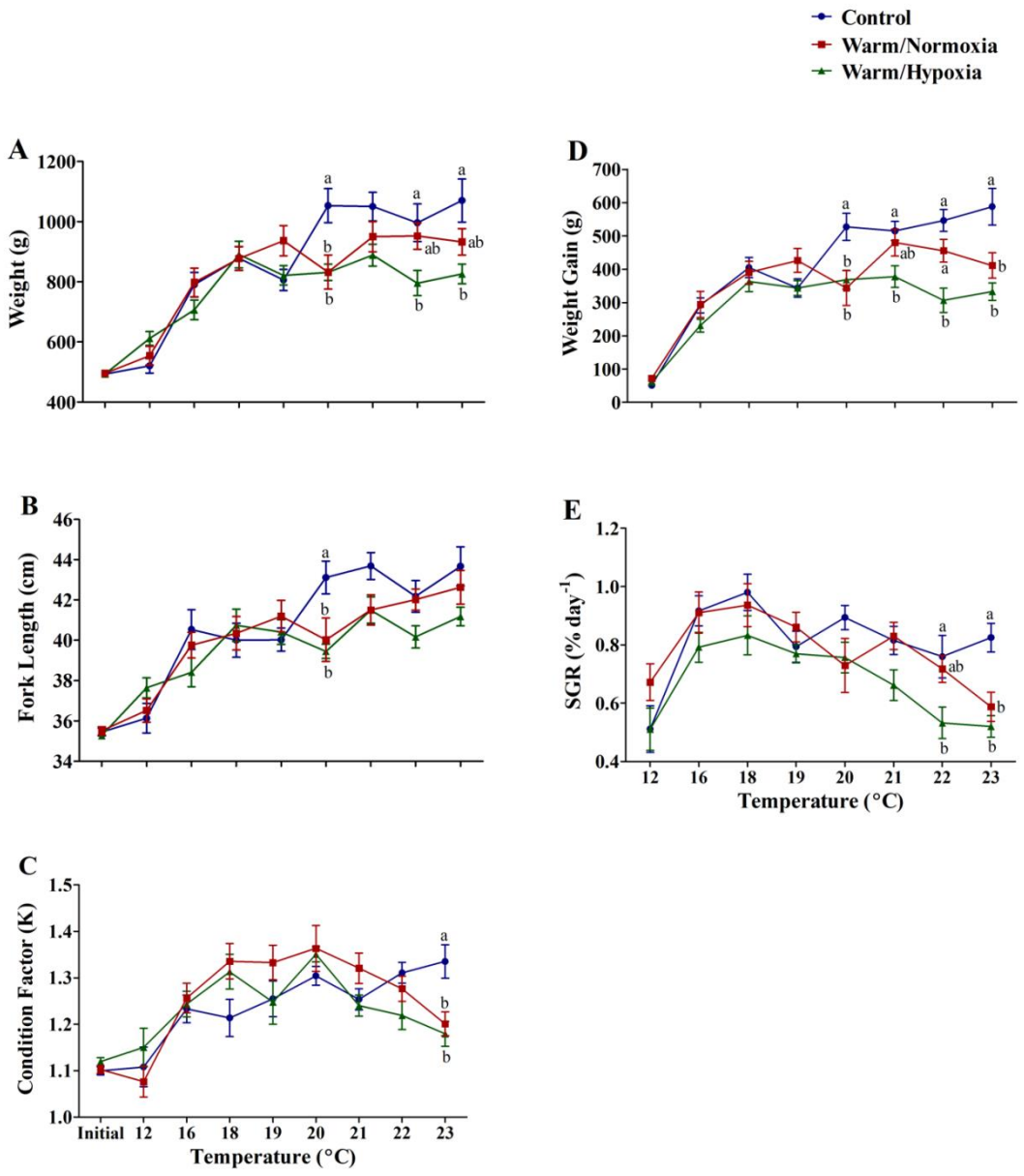

Figure 2.7. The effect of an incremental temperature increase alone (Warm/Normoxia), or in combination with moderate hypoxia (70% air saturation; Warm/Hypoxia) on the growth performance of Atlantic salmon (N = 10). The experiment was stopped at 23°C as mortality in these two groups had reached ~ 30%. Control fish were exposed to a constant temperature of 12°C and normoxia. Significant differences between the groups at each temperature are indicated by different letters ($P < 0.05$). Weight gain and SGR (panels D and E, respectively) at the temperatures shown were all calculated based on the initial weight of the fish in each group (see panel A). Values are means ± 1 S.E. (N = 10).

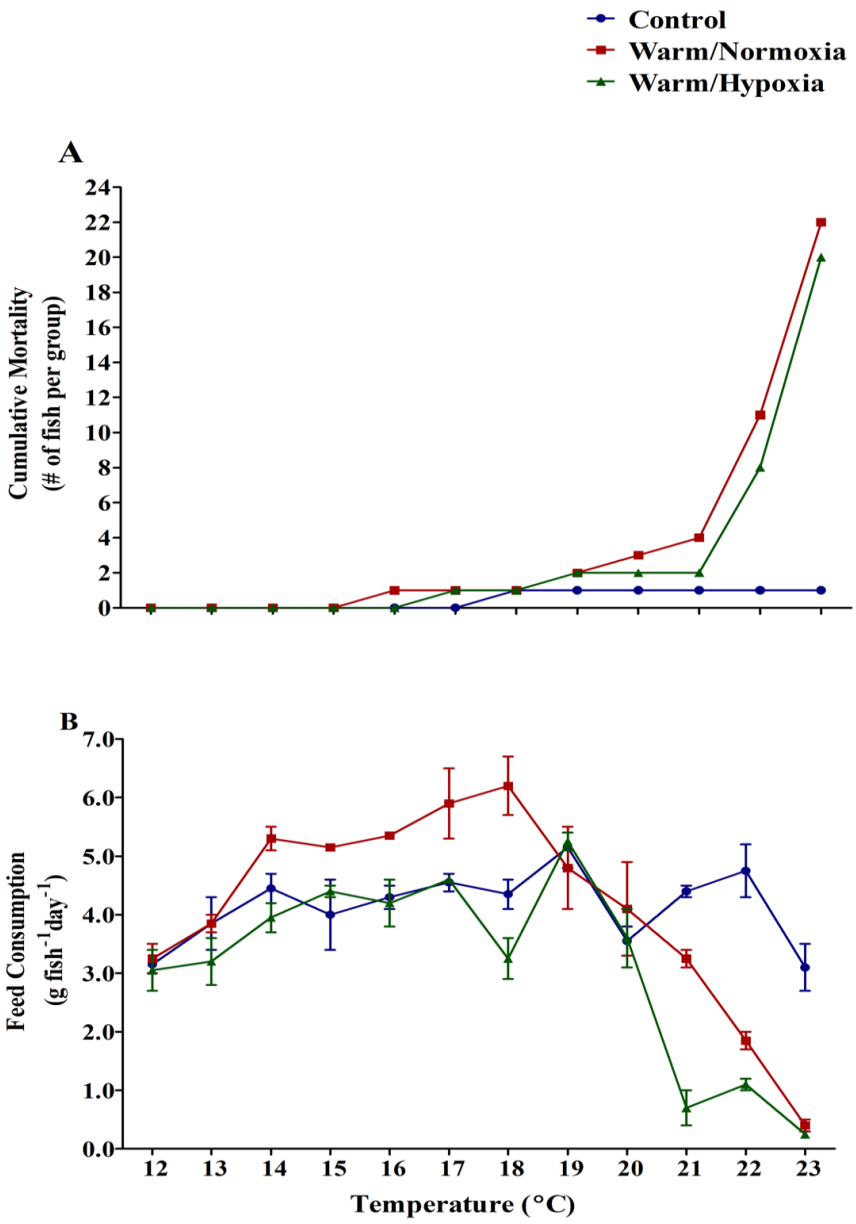

Figure 2.8. The effects of an incremental temperature increase alone (Warm/Normoxia), or in combination with moderate hypoxia (70% air sat.; Warm/Hypoxia), on cumulative mortality (A) and feed consumption (B) of Atlantic salmon. The experiment was stopped at 23^{o}C when mortalities in these two experimental groups reached ~ 30% (see results for further explanation of how cumulative mortality was estimated). Control fish were exposed to a constant temperature of 12^{o}C and normoxia. Values for feed consumption are means ± 1 S.E (N = 2 tanks), and represent the mean of 7 days at that temperature. No statistical analysis was performed because of the limited replication.

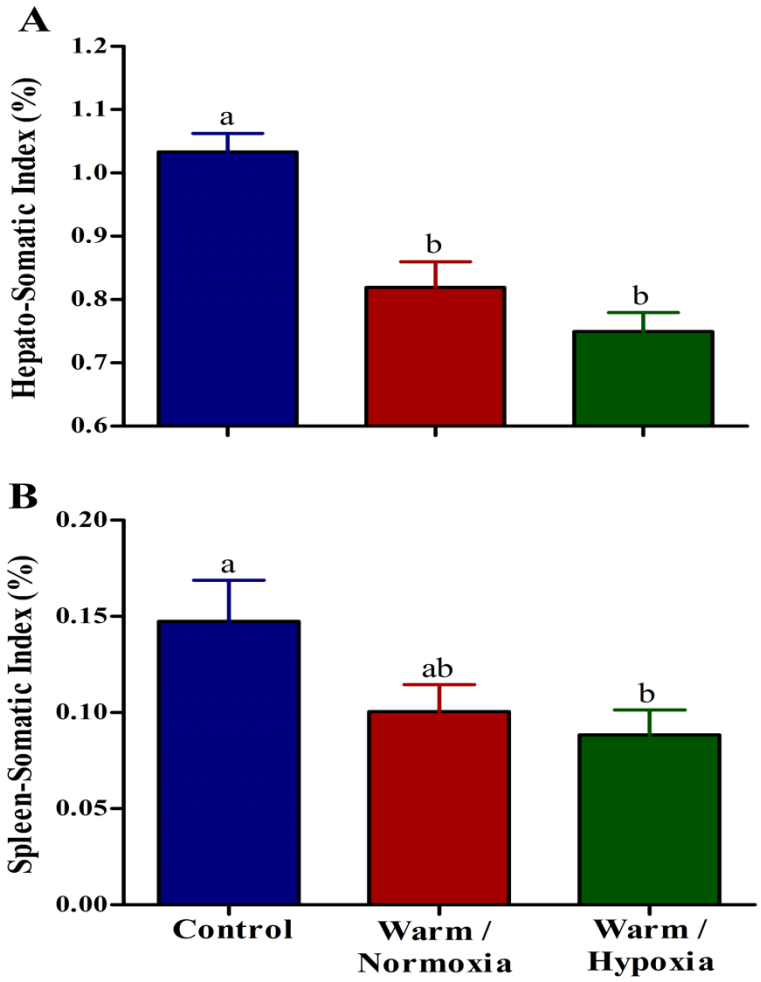

Figure 2.9. The effects of an incremental temperature increase alone (Warm/Normoxia), or in combination with moderate hypoxia (70% air sat.; Warm/Hypoxia), on the hepato-somatic index (A) and spleen-somatic index (B) of Atlantic salmon. The fish were sampled at 23^{o}C when mortalities in these two experimental groups reached ~30% (N = 10). Control fish were exposed to a constant temperature of 12^{o}C and normoxia. Significant differences ($P < 0.05$) between groups are indicated by different letters. Values are means ± 1 S.E.

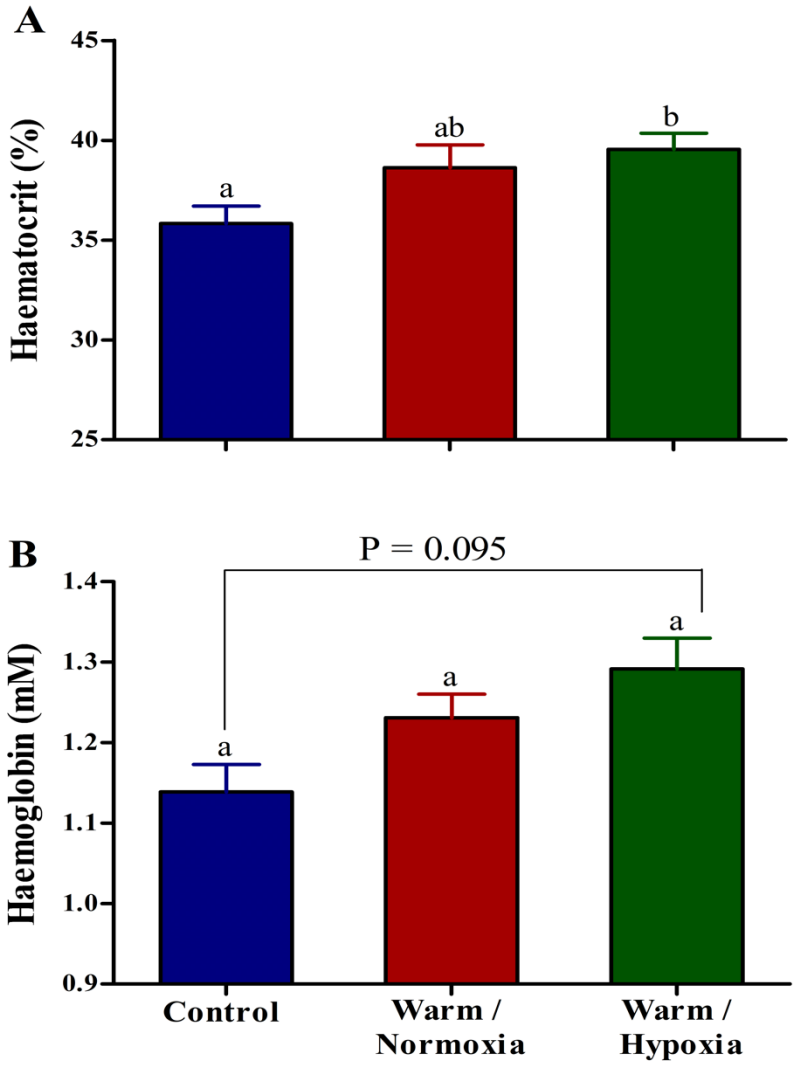

Figure 2.10. The effects of an incremental temperature increase alone (Warm/Normoxia), or in combination with moderate hypoxia (70% air sat.; Warm/Hypoxia), on the blood haematocrit (A) and haemoglobin concentration (B) of Atlantic salmon. The fish were sampled at 23^{o}C when mortalities in these two experimental groups reached ~30% (N = 10). Control fish were exposed to a constant temperature of 12^{o}C and normoxia. Significant differences ($P < 0.05$) between groups are indicated by different letters. Values are means ± 1 S.E.

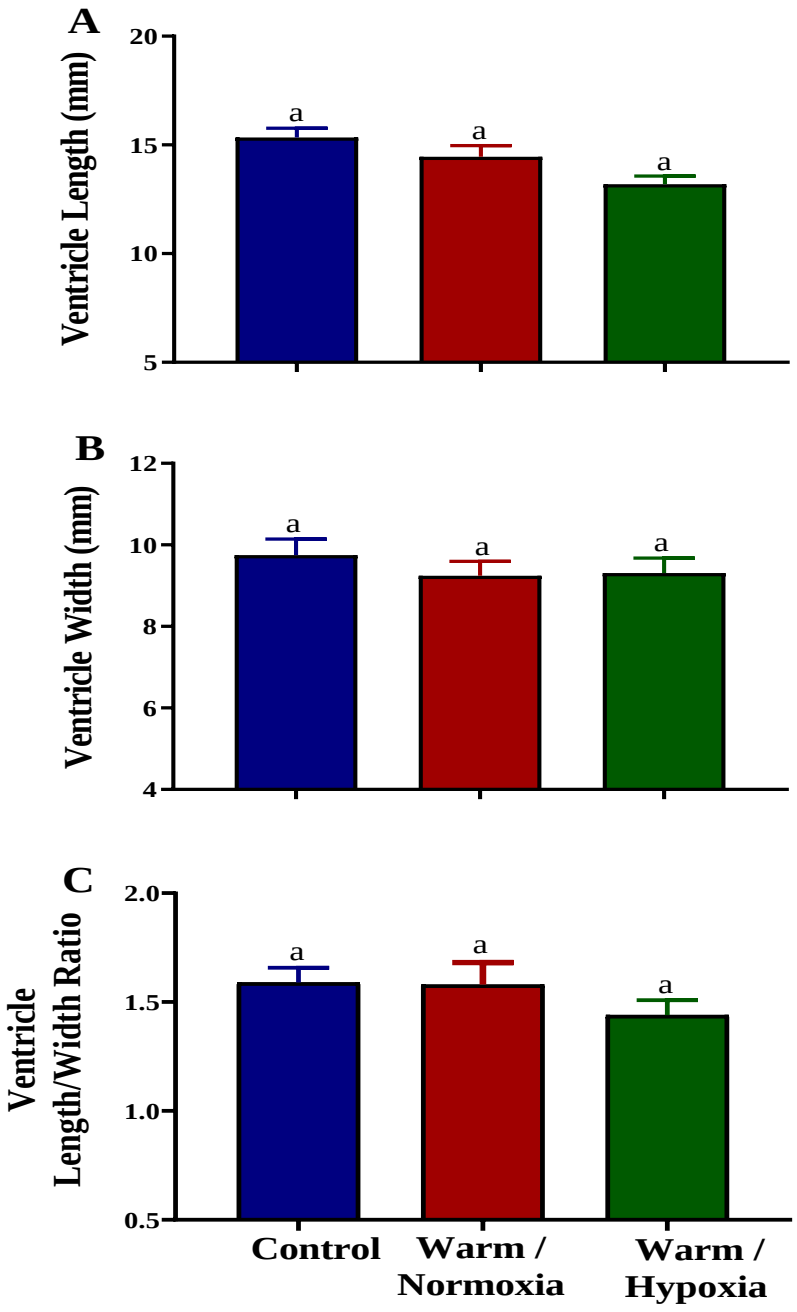

Figure 2.11. The effects of an incremental temperature increase alone (Warm/Normoxia), or in combination with moderate hypoxia (70% air sat.; Warm/Hypoxia), on ventricular length (A), ventricular width (B) and ventricular length:width ratio (C) of Atlantic salmon. The fish were sampled at 23^{o}C when mortalities in these two experimental groups reached ~ 30% (N = 10). Control fish were exposed to a constant temperature of 12^{o}C and normoxia. Values are means ± 1 S.E.

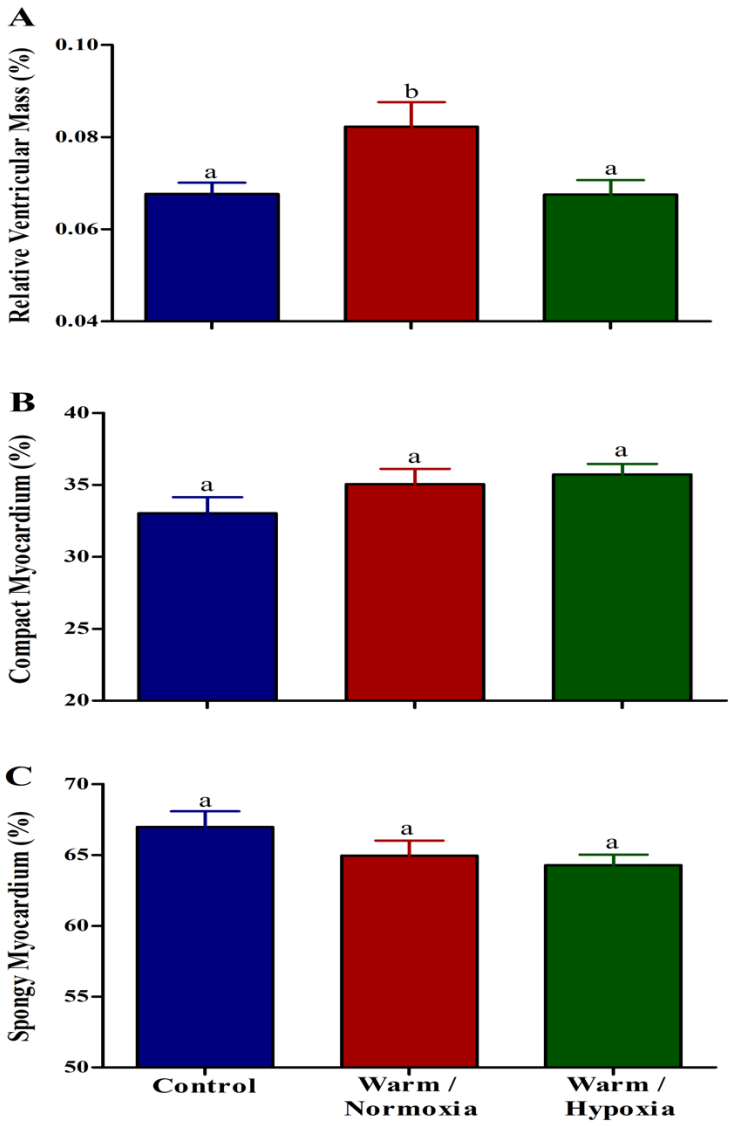

Figure 2.12. The effects of an incremental temperature increase alone (Warm/Normoxia), or in combination with moderate hypoxia (70% air sat.; Warm/Hypoxia), on the relative ventricular mass (A), % compact myocardium (B) and % spongy myocardium (C) of Atlantic salmon. The fish were sampled at 23°C when mortalities in these two experimental groups reached ~30% (N = 10). Control fish were exposed to a constant temperature of 12°C and normoxia. Significant differences ($P < 0.05$) between groups are indicated by different letters. Values are means ± 1 S.E.

4.0. DISCUSSION

4.1. Performance Metrics at 12^{o}C

The growth rate of the Control group at the start of Experiment $^{\#}$1 (i.e., for approx. the first month) at 12^{o}C was ~1.7 % day^{-1}, but then decreased to ~ 1.4 – 1.25 % day^{-1} at the end of the experiment (Figure 2.4E, Table 2.1). This initial growth rate at 12^{o}C is comparable to a number of studies on salmon of similar size held at 11-13^{o}C (Koskela et al., 1997; Kullgren et al., 2013; Hevrøy et al., 2015; Vikeså et al., 2015; Tromp et al., 2018). Growth rates in these studies ranged from ~1.14 to ~2.0% day^{-1}. Likewise, it was not surprising that the growth rate of this group decreased over the course of the experiment. SGR is known to decrease as fish get larger (Brett, 1979; Jobling, 1994; Koskela et al., 1997), and this decrease in SGR was also consistent with what was observed for feed consumption (FC). FC increased from the first to 3rd sampling and then plateaued, and when fish mass during the experiment is considered, feed consumption went from approx. 1% day^{-1} initially to approx. 0.65% day^{-1} at the end (Figure 2.5). Such a pattern of food consumption has previously been observed in salmonids held at 11-14 ^{o}C (Koskela et al., 1997; Hevrøy et al., 2012, 2013; Kullgren et al., 2013) and FC is also known to decrease with increasing fish size (Brett, 1979; Jobling, 1981; Coutant, 1987; Jobling, 1994; Koskela et al., 1997; Handeland et al., 2008).

4.2. Effects of the Incremental Temperature Increase

In this study an incremental temperature increase was employed that reflected summer / early fall temperature conditions in Atlantic Canada (Gollock et al., 2006; Burt et al., 2012), but also resembles those observed at some cage-sites in Europe (e.g.,

Bjornsson et al., 2007). Thus, the results are somewhat difficult to compare with the literature where salmon were generally held at constant temperatures for long periods (weeks to months; Handeland et al., 2008; Hevrøy et al., 2012, 2013, 2015; Kullgren et al., 2013; Vikeså et al., 2015; Nuez-Ortin et al., 2018; Tromp et al., 2018).

In the first study (Experiment #1), where fish were fed to encourage maximum growth (i.e., twice daily to satiation), the pattern of changes in SGR in the Warm/Normoxia group was similar to that observed in the Control (12^{o}C) group, and SGR, weight gain, condition factor etc. were not significantly different over the course of the experiment (e.g., see Figure 2.4, Table 2.1). These data strongly suggest that current water temperature changes in Atlantic Canada during the summer / early fall are unlikely to have major negative impacts on the production of sea caged Atlantic salmon of Saint John River origin. In fact, there were only two indications that the incremental temperature regime had a negative effect on salmon production. First, while feed consumption was higher in the Warm/Normoxia group from 15 - 19^{o}C (and by ~ 7% overall, Table 2.1), FC in the Warm/Normoxia group fell as temperature increased further and was not different from the Control group by the end of the experiment (Figure 2.5). This result is consistent with the data in Experiment #2, where FC also began to fall after 18^{o}C and rapidly declined as temperature was increased to 23^{o}C (Figure 2.8B), and with many studies on Atlantic salmon where fish were held at constant temperatures from ~ 10 – 19^{o}C. These authors reported that FC decreased at higher temperatures (Handeland et al., 2008; Hevrøy et al., 2012, 2013; Sambraus et al., 2017) and suggested that 18-19^{o}C is the upper limit for FC in salmon of the size range used in the present study. The decrease in feed consumption at higher temperatures may have been partially related to fish size, as

the optimum temperature for maximum feed consumption decreases as salmon get larger (Brett, 1979; Coutant, 1987; Jobling, 1981, 1994; Koskela et al., 1997). However, it is very likely that the decrease in FC was also related to a direct effect of temperature on appetite. There are several possible explanations for this decrease in appetite (feed consumption). A number of authors have suggested that FC is decreased at high temperatures because the salmon do not have sufficient aerobic scope to support the digestion and assimilation of feed (e.g. Hevrøy et al., 2012, 2015; Hansen et al., 2015), and the supply of oxygen for digestion is thought to control voluntary feed intake (Dam and Pauly, 1995). However, recent data have cast significant doubt on whether reductions in aerobic scope are responsible for (related to) the decrease in salmon feed consumption and growth at temperatures of 18^{o}C and above. Hvas et al. (2017) showed that the aerobic scope of Atlantic salmon (~ 450- 500g) held at temperatures from 5-23^{o}C was not significantly different when measured at 13, 18 and 23^{o}C. Instead, considerable research has been done on the control of appetite / FC in salmon at high temperatures, and it is probable that increases in the anorexigenic hormone leptin, and/or decreases in the orexigenic hormone ghrelin, mediated the decrease in feed consumption (Hevrøy et al., 2012, 2013, 2015; Kullgren et al., 2013).

The other consequence of the incremental rise in temperature was that Warm/Normoxia fish had a higher feed conversion ratio (~ 1.07) as compared to the Control group (~ 0.97), and this resulted in comparable growth despite their higher feed consumption (Figure 2.5; Table 2.1). This increase in FCR at high temperatures (i.e., > 18^{o}C) has been reported by some (e.g., Hevrøy et al., 2013; Kullgren et al., 2013) but not all (Hevrøy et al., 2015; Vikeså et al., 2015) authors, and may be size related (i.e., small

salmon are more tolerant of high temperatures; Handeland et al., 2008). The decrease in FCR in this study was undoubtedly associated with the effect of increasing temperature on the metabolic rate of the salmon. For example, measurements of Atlantic salmon routine oxygen consumption in respirometers, or in their holding tanks, show that this parameter increases significantly (i.e., approximately doubles) between 12 and 18-19^{o}C (Remen et al., 2013, 2016; Hevrøy et al., 2015; Hvas et al., 2017; Sambraus et al., 2017; Bowden et al., 2018). However, an increase in metabolic rate is probably not the only factor that led to the decrease in the FCR of salmon exposed to the incremental temperature increase. Energy losses in feces and excretory products also increase markedly with temperature (Elliott, 1991; Dockray et al., 1996).

Although the SGR of the salmon exposed to the incremental temperature increase was comparable to that of fish maintained at 12^{o}C in Experiment $^{\#}$1 (see Table 2.1), it is clear from Experiment $^{\#}$2 and the literature that increases in temperature beyond 20^{o}C are likely to negatively affect the growth and condition of the stocks used in these experiments. In Experiment $^{\#}$2, the SGR of the Warm/Normoxia group began to decrease relative to the Control (12^{o}C) group after 21^{o}C, and condition factor and HSI were ~ 8% and 21% lower, respectively, in 23^{o}C vs. 12^{o}C (Control) fish at the end of the experiment (Figures 2.7C and 2.9A). Further, while there were few mortalities up to 21^{o}C (i.e. 2-4 fish in total), 6-7 fish died in the two experimental groups at 22^{o}C and another 11-12 fish died at 23^{o}C (cumulative mortality ~ 15 and 30%, respectively) (Figure 2.8A). Indeed, these results are very similar to those of Hvas et al. (2017). These authors reported that Atlantic salmon acclimated to 23^{o}C began dying after 2 weeks at this temperature, that cumulative mortality reached 20% by 4 weeks, and that the fish would not eat at this

temperature and had a poor condition factor (i.e., ~ 0.87 vs. ~ 1.0 at the other acclimation temperatures). As mentioned previously, compromised aerobic scope cannot explain the mortality of these fish as this parameter was the same (if not slightly higher) at 23^{o}C vs. 15 and 18^{o}C. Hvas et al. (2017) suggested that the observed mortalities at 23^{o}C may have been associated with 'hyperactivity initiated as a form of escape behaviour'. Similar behaviour was observed in the current study, and thus, it is likely that whether fish are proactive or reactive in responding to high temperatures may strongly influence their tolerance limits. This hypothesis would be concordant with research on fish (including salmon) which have identified that these two behavioural traits influence hypoxia tolerance, with reactive fish characterized as having lower post-stress cortisol levels and better tolerance of low oxygen levels (van Raaij et al., 1996; Laursen et al., 2011; Damsgård et al., 2019). Indeed, in this study, cortisol values in the two experimental groups at 23^{o}C ranged greatly (i.e., 8 out of the 20 fish had levels < 10 ng mL^{-1}, whereas others had levels as high as 200 ng mL^{-1}; Zanuzzo et al., unpubl). Thus, it is possible that thermal tolerance in salmon and other fish species under culture conditions is more dependent on behaviour (i.e., the temperature at which fish evoke an escape response) than direct physiological / biochemical changes that result in cumulative stress levels that exceeded tolerable limits (Bevelhimer and Bennett, 2000).

As highlighted by Hvas et al. (2017) and studies of growth performance in Atlantic salmon, while the negative effects of high temperature may not be observed initially, they can have significant long-term impacts; i.e., the effects are time-dependent. For example, a number of authors (e.g., Handeland et al., 2008; Hevrøy et al., 2013, 2015; Vikeså et al., 2015) have shown that while SGR and/or fish mass were not, or only

minimally, affected in Atlantic salmon over the first few weeks at 18-19^{o}C as compared to Control fish held at 10-13^{o}C, substantial decreases in SGR (i.e., from ~ 15 – 50%) were reported when fish were held at these elevated temperatures for periods of a month or more. Further, this decrease in growth rate appeared to be related to the effects of exposure time at high temperatures on feed consumption. Remen et al. (2016) showed that daily feed intake was elevated in salmon held at 19^{o}C for the first 3 weeks at this temperature, but decreased thereafter, and was not different as compared to 15^{o}C acclimated fish by 3 weeks. Kullgren et al. (2013) reported that while feed intake of fish at 18^{o}C was equal to that of those held at 12^{o}C after 3 weeks, it gradually fell and was 27% lower than that recorded for 12^{o}C from approximately 6-7 weeks onward. Collectively, these data suggest that more prolonged exposure to temperatures of approx. 20^{o}C, or temperatures that exceed this level, could have significant negative effects on salmon production in Atlantic Canada. This is of particular concern as global water temperatures are predicted to increase over the next century by 1.5–2 °C (e.g., IPCC, 2018), and Dockray et al. (1996) showed that a simulated global warming scenario (i.e., the addition of 2^{o}C to the summer temperature profile of the Lake Ontario shoreline) resulted in depressed appetite and growth rate in juvenile rainbow trout. This was likely due to the fact that maximum temperatures were not only higher, but were above the threshold for optimal growth / food consumption for a longer period.

While the results of the current research are generally consistent with the studies described above (i.e., on European / Norwegian salmon stocks), they are in contrast to two studies that have recently been conducted on Atlantic salmon stocks used for aquaculture and stocking in Tasmania / Australia. For example, Nuez-Ortin et al. (2018)

maintained approx. 1 kg salmon from Huon Aquaculture stocks at 21°C for approx. 6 weeks, and reported that while condition factor and HSI were slightly lower in 21°C vs. 15°C acclimated fish, there was no effect of acclimation temperature on feed intake or final weight. Also, Tromp et al. (2018) showed that Atlantic salmon from a farm in Victoria (starting weight approx. 70 g) grew just as well, and had a higher condition factor, when reared at 20°C for 99 days as compared to fish held at 12 and 16°C. Although the latter result may be partially related to the small size of the fish used (but see Handeland et al., 2008), this research highlights that data on the thermal biology of Atlantic salmon from Tasmania / Australia may not be directly transferable to other populations, and that research needs to be specific to the fish (stocks) that are being used for marine aquaculture in a particular region.

4.3. Hypoxia and Increased Temperature

4.3.1. Hypoxia at 12°C

In these experiments, salmon in the Warm/Hypoxia group were lowered to approximately 70% (daily range ~ 65 - 75%; see Figure 2.2C) air saturation over 1 week, and kept at this oxygen level for another two weeks before the first sampling at 12°C (see Figure 2.2). This oxygen level is very similar to that observed at sea cages off the Newfoundland coast (Burt et al., 2012) and in aquaculture operations in Norway (Oppedal et al., 2011a), and is what triploid salmon in Tasmania select in the summer as a compromise between warmer surface waters and severely hypoxic conditions at greater depths (Stehfast et al., 2017).

At the end of this period, the fish exposed to hypoxia in Experiment #1 (where fish were fed to satiation) were significantly smaller, and their weight gain was significantly lower, as compared to the Control and Warm/Normoxia groups. Further, this was reflected in their SGR, which while not significantly different, was ~ 1.3 vs. 1.7 % day^{-1} (Figure 2.4). This depressed growth at 12^{o}C is very similar to that reported by Vikeså et al. (2017) for 1.3 kg salmon exposed to ~ 65% air saturation at 12^{o}C for 4 months. However, it was not associated with lower food consumption (see Figure 2.5), and while this is a difference as compared to Vikeså et al. (2017) who reported an ~ 10% decrease in food consumption, it does support their finding that normoxic fish fed the same amount as hypoxia-acclimated fish (i.e., pair-fed fish) had a higher growth rate. Collectively, these two studies suggest that hypoxia has a direct negative effect on fish growth which is independent of feeding / appetite. This decrease in growth was likely related to a higher feed conversion ratio as this metric was increased in both this study (Table 2.1) and Vikeså et al. (2017). However, the role that fish metabolism might play in the decreased growth observed in these two studies is not clear. Neither acute (Oldham et al., 2019) nor chronic (Remen et al., 2013; Motyka et al., 2017) hypoxia has an effect on the standard or routine metabolic rate of salmonids, and the limiting oxygen saturation for Atlantic salmon at 12^{o}C is between 35 and 47% air saturation (Remen et al., 2013, 2016). Further, while several studies have looked at the relationship between growth rate, metabolic scope and hypoxia in fishes (e.g. Claireaux et al. 2000), metabolic scope was only measured in fish exposed to acute, not prolonged (i.e., weeks of) hypoxia, and the analysis of Chabot and Claireaux (2008) suggests that the metabolic scope of fishes at 70% air saturation would be ~ 80-90% of that under normoxia. Also, it does not appear

that the hypoxia-related decrease in growth was related to alteration in aspects of the salmon's growth axis. Vikeså et al. (2017) did not find any difference between hypoxia- and normoxia-acclimated salmon in circulating IGF-1 levels, or liver / muscle *ghr-1* and *igf-1* mRNA levels.

4.3.2. Hypoxia in Combination with Increased Temperature

As hypoxic fish were warmed from 12^{o}C, it became apparent that hypoxia was having a negative effect on the salmon's feed consumption. For example, feed consumption was approx. 30% lower than that of both the Control and Warm/Normoxia groups at 13^{o}C, and was consistently 30-40 % lower as compared to the Warm/Normoxia group over the course of the experiment. That hypoxia decreased the salmon's FC was not a surprise as these fish were growing slower, and many studies have shown that lowering water oxygen levels decreases feed consumption in salmon and other fishes (Remen et al., 2012a, 2016; Hansen et al., 2015; Vikeså et al., 2017 and references therein). Remen et al. (2016) reported that the dissolved oxygen level required for maximum feed consumption (DO_{maxFI}) was 53% air saturation at 11^{o}C and 65% at 15^{o}C. Thus, given the oxygen fluctuations observed in the current study (65-75%; Figure 2.2A), feed consumption was not expected to decrease prior to the salmon reaching this latter temperature. However, the DO_{maxFI} values calculated by Remen et al. (2016) were determined for fish exposed to cycling hypoxic conditions, and thus, the current data combined with that of Vikeså et al. (2016), where FC was 10% lower at 12^{o}C, suggest that the DO_{maxFI} is lower in fish exposed to constant vs. fluctuating oxygen levels.

That FC and SGR were consistently lower in the Warm/Hypoxia group, but showed the same pattern of change with temperature as seen in the Warm/Normoxia group in Experiments #1 and #2, suggests that the effects of these two environmental challenges was additive. While this may be the case for these parameters under the current experimental conditions, it is apparent that temperature and hypoxia can also have a confounding influence on production characteristics. For example, fish in the Warm/Normoxia group had a higher FCR than the Control group, and while hypoxia increased this parameter at 12°C (i.e., these fish had a much lower weight gain but the same level of FC; see Figures 2.4D and 2.5, respectively), the lower growth in the Warm/Hypoxia group as temperatures rose appeared to be solely related to their lower FC (i.e., FCR in the Warm/Normoxia and Warm/Hypoxia groups over the course of the experiment was similar; Table 2.1). This suggests that exposure to hypoxia in combination with high temperature somehow improved (i.e., lowered) FCR as compared to what might be expected based on the independent impacts of these two environmental stressors. Such an effect was not unexpected, as while Vikeså et al. (2017) reported higher FCR in hypoxic salmon at 12°C, both Vikeså et al. (2016) and Hansen et al. (2015) showed that exposure to hypoxia failed to alter the FCR of salmon held at temperatures of 17-19°C.

Both high temperature and hypoxia are oxygen limiting conditions, and thus, one might have expected that exposing salmon to both hypoxia and high temperatures would have resulted in increased mortality compared to the latter stressor alone. Indeed, when Hansen et al. (2015) exposed ~ 1.3 kg Atlantic salmon to hypoxia (70% air saturation) at 17°C, 15% of the fish died. However, in the current study, there was no difference in the

temperature at which mortalities began (i.e., ~ 21-22^{o}C) and the level of mortalities at 23^{o}C (~ 30%) (Figure 2.8A). This may be because the fish were exposed to hypoxia for a prolonged period (i.e., for weeks) before temperature increases began [as opposed to Hansen et al., 2015 where O_2 levels were lowered at 17^{o}C over 3 days], and the positive changes in cardiorespiratory physiology and likely other physiological systems seen in this group (see below). Nonetheless, the critical oxygen tension / limiting oxygen saturation (LOS) for normoxic-acclimated Atlantic salmon at 22^{o}C is ~ 63-67% saturation (Barnes et al., 2011; Remen et al., 2013), and thus ~ 70% air saturation might have been just above a level where increased mortalities might have been expected; at least until temperatures were increased further. The latter hypothesis is supported by the results of Remen et al. (2013) who showed that cycling hypoxia (to levels as low as 40% air saturation) did not change the LOS of salmon when held at 16^{o}C.

4.4. Effects of Increased Temperature and Moderate Hypoxia on Blood Oxygen Carrying Capacity and Cardiac Morphology

Oxygen delivery to the tissues has been associated with fish thermal and hypoxia tolerance (Wang and Overgaard, 2007; Pörtner and Farrell, 2008; Farrell, 2009; Pörtner, 2010; Anttila et al., 2013, 2015; Muñoz et al., 2018), and blood oxygen carrying capacity (i.e., Hct and Hb) and cardiac morphometrics were measured in Experiment $^{\#}$2 when mortalities in the two experimental groups reached ~30% (i.e., at 23^{o}C). Based on these previous data it might be expected that acclimation to temperatures near the salmon's upper maximum temperature tolerance would be associated with an enhancement of factors that improve oxygen supply and delivery. However, there is a paucity of data on

how long-term exposure to temperatures above the 'normal seasonal range' influence cardiac morphology or function (Keen et al., 2017) or the blood's carrying capacity for oxygen.

At 23^{o}C, Hct and Hb were not significantly different between the Control and the Warm/Normoxia groups (Figure 2.10). This finding is consistent with the data for Atlantic salmon acclimated to temperatures between 13 and 19 ^{o}C (Hevrøy et al., 2013, 2015), but in contrast to studies where salmonids were exposed to incremental increases in temperature. For example, Sambraus et al. (2017) showed that salmon exposed to an incremental temperature increase from 6 to 18^{o}C had lower values for haematocrit at the warmer temperature, and Dockray et al. (1996) reported that the Hct of rainbow trout juveniles decreased from ~ 40% to 32.5% when seasonal temperatures increased from approx. 18 ^{o}C to 24-26 ^{o}C. In contrast to measures of blood oxygen carrying capacity in fish only exposed to the incremental temperature increase, the Warm/Hypoxia fish showed ~ 10 and ~ 13% increases in Hct ($P < 0.05$) and Hb ($p = 0.095$). This is somewhat surprising as neither long-term acclimation to 40% air saturation at 12^{o}C, or exposure to cycling hypoxia (down to 40% air saturation) resulted in an increase in these parameters in rainbow trout or Atlantic salmon (Remen et al., 2012; Motyka et al., 2017; Harter et al., 2019). However, it is possible that exposure to 70% air saturation at high temperatures resulted in a decrease in blood PO_2 or oxygen content (as opposed to exposure to high temperature alone, e.g. see Steinhausen et al. (2008), and this was sufficient to stimulate haematopoeisis

Interestingly, cardiac morphology was only minimally affected by the experimental treatments. For example, while RVM was marginally greater in the

Warm/Normoxia vs. Warm/Hypoxia fish in Experiment #1 (P = 0.064; Figure 2.6) and in the Warm/Normoxia group as compared to the other two groups in Experiment #2 (Figures. 2.11 and 2.12), there was no change in ventricle shape or the % compact and spongy myocardium with either of the experimental treatments. Although previous studies have shown that cardiac shape (e.g., ventricular length to width ratio) differs between wild vs. hatchery salmonids (Poppe et. al., 2003), and that this metric is associated with the heart's pumping capacity (Claireaux et al., 2005), it does not appear that ventricular shape is affected by slow (incremental) temperature increases that approach the salmon's upper thermal limit. This finding is in agreement with the study of Motyka et al. (2017) who reported that while exposure to chronic hypoxia (40% air saturation) had a negative impact on temperature-dependent rainbow trout cardiac function it did not influence cardiac shape, and together, these studies suggest that ventricle shape is not 'plastic' in salmonids and unlikely to be influenced by exposure to environmental stressors. Based on the literature, both the lack of a change in the proportion of compact myocardium, and the increase in RVM, with the temperature increase were unexpected. For example, previous studies have shown that acclimation to temperatures between 8-12^{o}C vs. 15-18^{o}C results in an increase in the proportion of compact myocardium in the salmonid heart (Klaiman et al., 2011; Anttila et al., 2015; Keen et al., 2016), and RVM has been shown to not change (Klaiman et al., 2011; Hevrøy et al., 2013, 2015; Keen et al., 2016) or to decrease (Anttila et al., 2015) when fish are acclimated to temperatures between 15 and 19^{o}C. These data suggest that the salmonid heart's response to increased temperatures is dependent on the maximum temperature to which it is exposed, and that an increase in size (as opposed to an increase in the percent

or thickness of compact myocardium) is the most beneficial response with regards to maintaining or increasing cardiac function at high temperatures. Why this might be is not known, as an increase in compact myocardium has been hypothesized to compensate for the negative effect of warm temperatures on the pressure generating ability of the heart (Farrell et al., 1996; Klaiman et al., 2011, 2014) and it has been suggested that temperature-induced changes in the compact layer may be related to the degree to which the coronary circulation is required to provide oxygen to the working muscle (Keen et al., 2016). Further, simultaneously exposing salmon to hypoxia and high temperature in the current study did not result in any changes in cardiac morphometry as compared to the Control group, and thus, could be seen as antagonistic to the effect of high temperatures alone on cardiac remodelling. It is possible that reducing water oxygen levels to 70% of air saturation at high temperatures, and its resultant effects on blood oxygen levels, constrained cardiac growth / remodelling, and that increasing blood oxygen carrying capacity (see Figure 2.10) was the preferred option to enhance oxygen delivery to the tissues. Nonetheless, an increase in haematocrit would be expected to increase blood viscosity and blood pressure (Brill and Jones, 1994), and thus, put further work demands on the heart.

5.0. Summary and Perspectives

Salmon in sea cages face increasing temperatures and low oxygen conditions (Johansson et al., 2006; Oppedal et al., 2011a; Burt et al., 2012; Stehfast et al., 2017), that are only expected to worsen in the current era of accelerated climate change (IPCC, 2018). This study specifically examined how an incremental increase in temperature

alone, and in combination with moderate hypoxia (~ 70% air saturation), affected the production characteristics and aspects of blood oxygen transport of farmed Atlantic salmon of Saint John River stock. These results support other research on this species suggesting that 18-19^{o}C is the upper limit for FC (and thus growth), but also show that temperatures up to 20^{o}C (even for a number of weeks) have few detrimental effects on these salmon (i.e., with the exception of increased FCR). However, they also show that that temperature increases beyond this level (i.e., above 21^{o}C) can result in significant mortalities and the cessation of feeding, and that moderate hypoxia may be of significant concern under present climate / temperature conditions. For example, moderate hypoxia reduces the growth of fish at 12^{o}C, and results in decreased food consumption and growth at temperatures that would be considered optimal for this species.

While this research has provided several valuable pieces of information for the Canadian aquaculture industry, it has also highlighted important areas for future research. First, while the OCLTT concept (Pörtner et al., 2010, 2017) theorizes that upper thermal tolerance is primarily determined by aerobic scope, there was no difference in temperature-dependent survival between the Warm/Normoxia and Warm/Hypoxia groups. Given that 70% hypoxia should reduce aerobic scope by ~ 20% (Chabot and Claireaux, 2008), this lack of difference in survival supports the findings of Hvas et al. (2017) that Atlantic salmon have adequate aerobic scope to tolerate temperatures as high as 23^{o}C. Thus, based on these observations of salmon behaviour at high temperatures and the large variation in cortisol data at 23^{o}C [Zanuzzo et al., (in prep)], I it is likely that whether a fish's coping style is reactive or proactive may have a large influence on the temperature they can tolerate. Clearly, more research needs to be conducted on the

linkage between these two traits, especially since salmon possessing the reactive behavioural phenotype (i.e., those more tolerant of hypoxia and high temperature) appear to also have slower growth rates and later smoltification [Damsgård et al. (2019)]. This may make it difficult to select for high growth and tolerance to adverse environmental conditions simultaneously.

Second, while we have begun to comprehend how increasing temperature and hypoxia interact to influence fish biology and physiology (e.g., Anttila et al., 2013; McBryan et al., 2013; Ern et al., 2016; Motyka et al., 2017; Vikeså et al., 2017), it is apparent that we need a better understanding of the interactive, and potentially confounding (e.g., see Vikeså et al., 2017), effects of these two key environmental variables. For example: (1) Given that both high temperature and moderate hypoxia independently resulted in a decrease in FCR, I would have expected an even further reduction in FCR when hypoxia fish were exposed to increasing temperature. However, the FCR of the Warm/Normoxia and Warm/Hypoxia groups was the same over the course of Experiment $^{\#}$1. (2) Moderate hypoxia had no effect on the ability of Atlantic salmon to withstand an incremental temperature increase (see above). (3) Based on the comparison of these data with that of previous authors (e.g., Remen et al., 2016), it appears that the effects of cycling vs. constant hypoxia at elevated temperatures may be different. Finally, (4) high temperature vs. high temperature + hypoxia resulted in different responses of the cardiorespiratory system (i.e., cardiac hypertrophy vs. increased haematocrit, respectively), and research conducted simultaneously on fish in Experiment $^{\#}$1 showed that while hypoxia shifted the PCA clustering pattern of hepatic mRNA expression for 27 temperature, hypoxia and oxidative stress-related genes as compared to normoxic fish at

cooler temperatures (i.e., 12 and 16^{o}C), the distribution of gene expression in these two groups was very similar at the higher temperatures of 18 and 20^{o}C (Beemelmanns et al., in prep). Such research will be critical for understanding, and mitigating, the potential impact of these two co-occurring environmental stressors on the Atlantic salmon industry, and for conservation and management strategies with respect to wild populations of this species.

6.0. References

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