To What Extent Do Acute Exposure and Acclimation to Cold Temperatures Impact the Cardiorespiratory Capacity, Stress Response and Swimming Performance of Atlantic Salmon (*Salmo salar*)

by

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Abstract

Limited research has been conducted on the physiology of Atlantic salmon (*Salmo salar*) at cold temperatures despite significant winter mortalities at sea-cage sites in Atlantic Canada. Thus, I performed two experiments to investigate how acclimation to 8, 4 and 1°C, and acute cooling from 8-1°C, affected the Atlantic salmon's cardiac function, metabolic capacity, stress physiology and swimming performance. While this research shows that exposure to 1°C causes stress, and significantly reduces their metabolism and swimming performance, it also reveals that there is significant flexibility/plasticity in how salmon modulate heart function when acutely vs. chronically exposed to cold temperatures. For example, the experiments were performed on salmon from two different aquaculture companies, and changes in resting heart rate and size were only seen in 1°C acclimated fish in Chapter 3. This data suggests that responses to cold temperatures differ between cultured salmon populations. Further, how salmon meet the energetic demands of exhaustive exercise depends on the duration of cold exposure [e.g., fish acutely cooled to 1°C predominantly enhance tissue oxygen extraction, whereas those acclimated to this temperature increase stroke volume]. These studies add greatly to our understanding of how temperatures close to a salmon's (fish's) lower thermal limit impact its physiological capacity and the mechanisms involved.

General Summary

Climate change poses a significant threat to many important aquatic species, including wild fish species and those produced at aquaculture cage-sites in coastal regions. Summer temperatures due to rising average ocean temperatures and marine heat waves, and winter 'super-chill' events, have been linked to mortalities at Atlantic salmon cage-sites on Canada's east coast. However, there is very limited information available on how exposure to cold (~ 0-1°C) temperatures affects this species' physiology and their capacity to tolerate these temperatures. Therefore, I acclimated salmon to 8, 4 and 1°C, and exposed others to an acute drop in temperature from 8 to 1°C, and examined the effects on the salmon's metabolism, heart function, swimming performance and level of stress. Although cold temperatures are stressful for salmon and reduce their swimming performance, this species shows considerable plasticity in how they deal with cold temperatures. Further, salmon respond quite differently depending on whether the cold temperature exposure is chronic or acute (i.e., the duration of exposure), and it appears that population differences influence their ability to respond physiologically when exposed to such temperatures.

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This thesis is dedicated to my oldest sister who had an extreme passion for all marine life, including fish.

Co-Authorship Statement

The research presented in this M.Sc. thesis was performed by Emma Porter, under the supervision of Dr. A. Kurt Gamperl. Emma Porter shared in the design of the experiments and was primarily responsible for: the rearing and care of experimental animals; conducting the described experiments; collecting and analyzing the data; and the writing the first draft of all chapters of this thesis. This research was made possible through the collaboration of several key individuals.

Rebeccah M. Sandrelli[1] assisted in animal care and facilitated proper experimental design and setup, and provided edits for **Chapter 2**. Kathy A. Clow[1] provided extensive surgical assistance and training on the biochemical analyses performed in **Chapters 2 and 3**, and edited **Chapter 2**. Dr. A. Kurt Gamperl[1] provided supervision and integral input through the research program (including the identification of research topics and experimental design, support during data collection and analysis, and provided edits on all section of this thesis).

From this thesis, there will be two publications:

Authorship for the publication derived from **Chapter 2** is: Emma S. Porter, Kathy A. Clow, Rebeccah M. Sandrelli, A. Kurt Gamperl. This manuscript was published in the journal *Current Research in Physiology* on March 17, 2022. doi: 10.1016/j.crphys.2022.03.002

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°C	Degrees centigrade
AD	Adrenaline
ADP	Adenosine diphosphate
AbS	Absolute scope
AS	Aerobic scope
BSC	Brain-sympathetic-chromaffin cell
CA	Catecholamines
CT_{Max}	Critical thermal maximum
CVP	Central venous pressure
EDTA	Ethylenediamine tetraacetic acid
f_H	Heart rate
f_{Had}	Adrenergic tonus
f_{Hch}	Cholinergic tonus
f_{Hint}	Intrinsic heart rate
f_{HMax}	Maximum heart rate
FS	Factorial scope
Hct	Haematocrit
Hb	Haemoglobin
HPI axis	Hypothalamus-pituitary-interrenal axis
IPCC	Intergovernmental Panel on Climate Change
IT_{Max}	Incremental temperature maximum
K	Condition factor
MCHC	Mean cellular haemoglobin concentration
MMR	Maximum metabolic rate
$\dot{M}O_2$	Oxygen consumption
$\dot{M}O_2 / \dot{Q}$	Tissue oxygen extraction
MS-222	Tricaine methanesulfonate
NAD	Noradrenaline
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PO_2	Partial pressure of oxygen
PO_{max}	Maximum power output
ppt	Parts per thousand
$\dot{Q}$	Cardiac output
Q_{10}	The fractional change in a rate over a 10°C range

RMR	Routine metabolic rate
ROS	Reactive oxygen species
RVM	Relative ventricular mass
SAN	Sinoatrial node
SMR	Standard metabolic rate
U_{crit}	Critical swimming speed
V_S	Stroke volume

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CHAPTER 1: General Introduction

1.1 The Atlantic salmon

The Atlantic salmon (*Salmo salar*) is an economically and ecologically important salmonid species distributed throughout the North Atlantic (Holm, 2000; Lacroix & Knox, 2005). Wild salmon hatch in freshwater rivers, migrate as smolts to the ocean, and return to their natal rivers to reproduce at sexual maturity (Jonsson & Jonsson, 2006). Thus, as part of their life cycle, they migrate long distances and must tolerate many environmental changes (i.e., in water velocity, temperature, dissolved oxygen and salinity). For example, they encounter many different thermal habitats, including shallow rivers, estuaries, fjords, coastal marine areas and the open ocean, and may experience temperatures as low as 0ºC and as high as 28-30ºC (Corey et al., 2017; Holm, 2000; Jacobsen et al., 2012; Jacobsen & Hansen, 2001; Moore et al., 2012; Strøm et al., 2020). Historically Atlantic salmon were an important wild fishery in many countries, but moratoriums on the harvesting of wild salmon are in place in most regions, and reported global landings were a mere 888 metric tonnes (MT) in 2020, a 93% reduction since the estimated peak in 1967 (The North Atlantic Salmon Conservation Organization NASCO, 2021). In contrast, this species is now the fourth most economically important finfish aquaculture species globally, behind carp, tilapia and catfishes, with worldwide production increasing by ~ 10.2% from 2.44 million metric tonnes (MMT) in 2010 to 2.69 MMT in 2019 (Tveteras et al., 2019).

The importance of this species for aquaculture production is not surprising given the large number of countries that have a significant Atlantic salmon aquaculture industry (i.e., Norway, Chile, Canada, Scotland, the Faroe Islands, Australia, Iceland, the United States, Ireland, New Zealand etc.) and its high market price (Iversen et al., 2020). Although salmon production continues to grow globally, regional declines have been reported, including in many parts of Canada. For example, the production and market value of Atlantic salmon on the East Coast of

Canada (i.e., in Newfoundland) decreased by ~ 65% from 2016 to 2020 (Seafood Industry Year in Review SIYIR, 2020). There are several reasons for this significant decline, including problems with sea lice, mass mortality events and COVID-19 global market challenges, yet many questions remain unanswered/unexplored, and thus, this topic has garnered significant attention from the media and the scientific community (Beemelmanns et al., 2021a,b; CBC News Newfoundland, 2020; Ćirić, 2020; Gamperl et al., 2020, 2021; Gerber et al., 2021a,b; Huffman, 2019; Hvas et al., 2017; Liu et al., 2020; The Canadian Press, 2015).

The use of land-based culture systems for salmon production has increased in the past decade due to significant technological advancements in recirculating water systems, genetic selection and disease control/mitigation (Devlin et al., 2020; Ignatz et al., 2020; Lhorente et al., 2019; Saunders et al., 1998). However, the majority of salmon production still takes place in coastal areas, and uses net-pens or "sea-cages" to rear salmon from smolts to market size. Although there are many advantages of sea-cage production, including significantly less energy usage for facility operations, the unpredictability of weather/climatic shifts (whether acute or chronic) does not come without a cost to animal health and welfare / survival (Ibieta et al., 2011; Weitzman & Bailey, 2019). Salmonid cage-site management is continuously being modified to enhance production, including changes in cage depth and design, augmentation of water flow/exchange, site selection, oxygen supplementation, pathogen control and mitigation, and on-line environmental monitoring. However, a number of challenges still exist (Falconer et al., 2020; Reid et al., 2019). For example, harsh weather events create unexpected problems, including changes in dissolved oxygen, salinity and temperature (Burt et al., 2012; Harley et al., 2006; Pörtner, 2010; Pörtner & Knust, 2007; Stehfest et al., 2017). Abrupt and long-term changes to the environment would typically result in behavioural avoidance by wild salmon (Breau et al., 2011; Gallant et al.,

2017). However, this is not possible when salmon are held in moored cages that are typically secured in place in coastal waters and are only 15 – 30 m deep (Olsvik et al., 2013). Lethal and sublethal impacts of various environmental stressors on salmon physiology, welfare, and production, are therefore, of great interest to the aquaculture industry, and understanding how to mitigate them will become increasingly relevant given climate change projections for the next several decades (Breitburg et al., 2018; Frölicher et al., 2018; Gamperl et al., 2020; Gerber et al., 2021b; IPCC, 2018, 2022; Oliver et al., 2018; Tromp et al., 2018; Wade et al., 2019).

1.2 Impacts of a changing climate

The Intergovernmental Panel on Climate Change (IPCC) has released several alarming reports over the past 20 years that synthesize comprehensive scientific data sets collected from around the globe. These reports provide compelling evidence that the rate and severity of harmful/challenging climatic events is increasing, and suggest that this poses serious risks to the world's ecosystems; including major impacts on aquatic/marine coastal environments. The IPCC is currently predicting a rise in average global sea-surface temperatures of 1.5 to 4.5°C at high latitudes, which we now know should not exceed 1.5°C to minimize the effects on coastal marine species (IPCC, 2018, 2022). The potential impacts of high seawater temperatures on the salmon aquaculture industry is exemplified by the maximum temperatures (22.9 to 24.2 ^{o}C) currently being observed in sea-cages at 5 to 20 meters depth in Tasmania (Stehfast et al., 2017;Wade et al., 2019), and the loss of 2.6 million salmon in the summer of 2019 in Newfoundland, Canada. This mortality event was reported to have been caused by several interacting factors, including water temperatures greater than 18^{o}C for 117 days (Burke et al., 2020), and it has been predicted that summer temperatures on the south coast of Newfoundland, where the majority of salmon farming

takes place in the province, will increase by 3.0°C by the middle of the century (Government of Newfoundland and Labrador, 2018). Nonetheless, coastal water temperatures undergo considerable variation seasonally, and with depth, at sea-cage sites both in Europe (Björnsson et al., 2007a,b) and on Canada's east coast (Gollock, 2006; Norin et al., 2019), with winter temperatures typically reaching 0 to 2°C for extended periods (Cooke Aquaculture Inc., Pers. Comm; Vadboncoeur et al., 2023). Further, daily drops in sea surface temperatures from 16 to 2-3°C at ~ 1°C h^{-1} can also be observed during the summer months in coastal sea-cages at 10 m depth (Gollock et al., 2006; Strøm et al., 2020), and are often due to surface waters turning over during periods of high winds and rough oceanic conditions (Donaldson et al., 2008; Wang et al., 2010).

As climate change intensifies, it is expected that weather extremes will be reoccurring events, and that these will result in more frequent and extreme marine heatwaves as well as severe reductions in ocean water temperatures (i.e., episodes of 'cold shock') (Johnson et al., 2018; Szekeres et al., 2016). Indeed, winter 'cold shock' events caused large-scale losses of Atlantic salmon at sea-cages in Atlantic Canada in 2014, 2015 and 2019 (termed 'winter kill') (CBC News, Newfoundland, 2020; Edwards, 2020; Evans, 2019; Huffman, 2019). These climate-driven impacts are a growing concern for salmon farmers in Atlantic Canada, and are being prioritized as a key research area. Indeed, there are many studies and publications describing the effects of high temperatures on salmon physiology, production and mortality (Anttila et al., 2014; Beemelmanns et al., 2021a,b; Gamperl et al., 2020, 2021; Gerber et al., 2021a,b; Jutfelt, 2020; Keen & Gamperl, 2012; Penney et al., 2014; Tromp et al., 2018). In contrast, there are only a handful of studies that document the effects of cold temperatures on cultured salmonids (Donaldson et al., 2008; Liu et al., 2020; Sandnes et al., 1988), despite it being established almost five decades ago that the lower

temperature limit for salmonids (including Atlantic salmon) in seawater is ~ 1°C based on poor growth and the risk of mortality (Saunders et al., 1975). Importantly, information is needed to understand: 1) how the distribution and behavior of salmon are impacted by exposure to cold temperatures at sea-cage sites (i.e., under 'real-world' aquaculture conditions); 2) at what temperature sub-lethal effects occur in Atlantic salmon and their severity; and 3) how cold temperatures impact the physiology and welfare of Atlantic salmon.

1.3 Temperature, cardiorespiratory function and swimming performance

Temperature has been described as the 'ecological master factor' in aquatic ectotherms, including fishes, as it affects numerous biological functions and metabolic activities through changes in the rate of biochemical reactions, and these have downstream impacts on various physiological processes (Brett, 1971). As previously mentioned, a large body of research has focused on the effects of rising ocean temperatures and marine heatwaves (i.e., prolonged and acute increases in water temperature) on salmon physiology. For example, there have been several comprehensive studies that have examined the effects of increased water temperatures on salmonid (including Atlantic salmon) physiology and biology, and their capacity to respond to these challenges via phenotypic plasticity (i.e., acclimatory responses) and evolutionary adaptation (i.e., through changes in genetics) (Adams et al., 2022; Anttila et al., 2014; Beemelmanns et al., 2021a, b; Chen et al., 2018; Gallant et al., 2017; Gamperl et al., 2020; Gerber et al., 2020 a, b; Jutfelt, 2020; Penney et al., 2014; Sandblom et al., 2016; Tromp et al., 2018). This type of research has involved measurements of upper thermal tolerance [i.e., critical thermal maxima (CT_{max}) and incremental temperature maxima (IT_{max})] in parallel with cardiorespiratory parameters such as oxygen consumption ($\dot{M}O_2$), cardiac output ($\dot{Q}$), heart rate (f_H), stroke volume (V_S), blood oxygen

transport, and tissue oxygen extraction ($\dot{M}O_2$ / $\dot{Q}$) (Anttila et al., 2014; Ekström et al., 2019; Gollock, 2006; Hvas et al., 2017; Motyka et al., 2017; Penney et al., 2014). These measurements/parameters have been a primary focus of numerous studies as heart function and oxygen delivery to the tissues are throught to be two of the principal factors that determine the upper thermal tolerance of fishes (Farrell et al., 2009; Franklin et al., 2013; Iftikar & Hickey, 2013; Jutfelt et al., 2018; Pörtner, 2010; Wang & Overgaard, 2007).

In addition to research on fish approaching their upper thermal limits, there has been a considerable body of literature describing the effects of cold temperatures on the cardiorespiratory physiology of fishes. However, this research has predominantly focused on Antarctic/polar species (Farrell & Steffensen, 2005). Further, most of the *in vivo* studies on temperate species at low temperatures/following acclimation to cold temperatures has focused on increases in heart size (i.e., relative ventricular mass, RVM) and other aspects of cardiac remodelling (Aho & Vornanen, 2001; Driedzic et al., 1996; Eliason & Anttila, 2017; Johnson et al., 2014; Keen et al., 2017; Klaiman et al., 2014), and there are very few measurements of heart function at temperatures approaching 0ºC (Costa et al., 2013, 2015; Farrell et al., 2013; Franklin et al., 2013; Lurman et al., 2012). Importantly, there is little to no information on how: 1) exposure to these temperatures affects the contribution of intrinsic heart rate, adrenergic and cholinergic nervous innervation (tone), and/or circulating catecholamines to the control of heart function in non-Antarctic fishes; 2) changes in cardiorespiratory function and/or capacity may, at least partially, compensate for the effects of temperatures approaching the lower thermal limit for salmonids; and 3) the swimming performance of Atlantic salmon is impacted by acute and chronic exposure to temperatures approaching this species' lower limit. Increases in f_H and V_S (and therefore $\dot{Q}$) have been described for salmonids when swum to exhaustion at both optimal and higher temperatures (Claireaux et al.,

2005; Farrell & Smith, 2017). However, to my knowledge there are no measurements of salmonid/North Atlantic fish swimming performance and cardiac function at 0-1^{o}C, and it is unclear how long-term exposure to these temperatures will affect the swimming or aerobic capacity of temperate fishes. A study by Riseth et al. (2020) reported that the aerobic scope (AS) and critical swim speed (U_{crit}) of 3^{o}C-acclimated Atlantic salmon were approximately 45 and 30% lower, respectively, as compared to conspecifics acclimated to 10.5 ^{o}C, while Claireaux et al. (2000) found a more drastic reduction (by 50 and 64%) in aerobic scope of Atlantic cod when acclimated to 2^{o}C vs. 5 and 10°C, respectively. In contrast, Hvas et al. (2017) showed that the U_{crit} of Atlantic salmon only decreased by 12% when acclimated to 3 vs 8 ^{o}C, and these data are comparable to the findings of Taugbøl et al. (2019). These authors showed that the swimming performance of brown trout (*Salmo trutta*) was also only marginally lower (by ~ 13%) when acclimated and tested at 1.7 vs. 5.5^{o}C.

In conclusion, examining these physiological questions is particularly important given the low temperatures experienced by Atlantic salmon in the wild and in culture, the predicted increase in the frequency of 'cold events', and that sufficient swimming performance and cardiorespiratory capacity are critical for many important biological functions; including migration/movement, feeding, digestion and predator-prey interations. By determining how physiological parameters such as stress, heart function, blood oxygen delivery and swimming performance are impacted by cold temperatures, we will be able to better predict, and model, salmon distribution, behaviour and health/welfare in both sea-cages and in their natural environment. Thus, the following chapters examine how both acute and chronic exposure of Atlantic salmon to extremely low/cold temperatures (i.e., 1^{o}C) impact multiple aspects of this species' physiological capacity.

1.4 Goals and objectives

Given: the negative impacts that cold temperatures have on Atlantic salmon production and welfare; the fundamental research questions that remain about how temperatures approaching 0^{o}C affect the cardiorespiratory and swimming performance/capacity of salmonids; and that heart size and function are related to inter-family differences in the hypoxia and upper thermal tolerance of Atlantic salmon (Anttila et al., 2013) - suggesting this trait (heart size) could be selected for in breeding programs - this thesis asked several very important and relevant questions:

1. What effect does an acute temperature drop from 8 to 1^{o}C have on heart function and control, oxygen consumption and stress hormone levels in Atlantic salmon?
2. To what extent are the above temperature-dependent changes ameliorated / altered when salmon are allowed to acclimate to 1^{o}C; i.e., what is the salmon's phenotypic plasticity to cope with cold water exposure?
3. How is the salmon's cardiorespiratory and aerobic swimming capacity affected by acclimation (8, 4 and 1^{o}C) and acute exposure (from 8 to 1^{o}C) to cold temperatures?

This important research was conducted at the same time as measurements of the heart rate (electrocardiograms; ECGs), activity, temperature and depth of Atlantic salmon in sea-cages during the winter on Newfoundland's south coast (R. Sandrelli, M.Sc. thesis), and of biomarkers of sub-lethal cold stress in salmon (E. Vadboncoeur, M.Sc. thesis). Interestingly, R. Sandrelli found that although sea surface temperatures during the winter months (i.e., from mid March to mid May) were ~ 2°C, salmon held in sea-cages typically stayed within the top 5 m of the cage with only periodic migrations to deeper/warmer waters. In addition, E. Vadboncoeur showed that

exposing salmon to cold temperatures (< 8°C) in a lab-based setting resulted in: 1) a decline in appetite, with complete cessation at 1-2°C; 2) changes in osmoregularity capacity; 3) an increased expression of heat shock proteins (at 4-5°C); 4) elevated levels of stress hormones (i.e., cortisol); and 5) symptoms indicative of 'Winter Syndrome' such as enlarged livers and ulcers on the head/jaw. Collectively, this research greatly enhances our knowledge of the biology and physiology of Atlantic salmon (salmonids) at cold temperatures and provides key/critical information to the salmon aquaculture industry. This includes data on the welfare of their fish at cold temperatures and information on how to manage their farms during the winter months. Alternatively, they could initiate additional vaccine programs, incorporate functional feed ingredients into their diets, and/or use netting to restrict salmon to deeper sections of the cage when sea-surface temperatures reach critical limits.

1.5 References

Adams, O. A., Zhang, Y., Gilbert, M. H., Lawrence, C. S., Snow, M., Farrell, A. P., Cooke, S., 2022. An unusually high upper thermal acclimation potential for rainbow trout. Conserv. Biol. 10(1), coab101. https://doi.org/10.1093/conphys/coab101

Aho, E., Vornanen, M., 2001. Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B. 171(2), 173–179. https://doi.org/10.1007/s003600000171

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R., 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. J. Exp. Biol. 216(7), 1183–1190. https://doi.org/10.1242/jeb.080556

Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E., Farrell, A. P., 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. Nat. Commun. 5(1), 4252. https://doi.org/10.1038/ncomms5252

Beemelmanns, A., Ribas, L., Anastasiadi, D., Moraleda-Prados, J., Zanuzzo, F. S., Rise, M. L., Gamperl, A. K. 2021a. DNA methylation dynamics in Atlantic salmon (*Salmo salar*) challenged with high temperature and moderate hypoxia. Front. Mar. Sci. 7, 604878. https://doi.org/10.3389/fmars.2020.604878

Beemelmanns, A., Zanuzzo, F. S., Xue, X., Sandrelli, R. M., Rise, M. L., Gamperl, A. K., 2021b. The transcriptomic responses of Atlantic salmon (*Salmo salar*) to high temperature stress alone, and in combination with moderate hypoxia. BMC. Genomics. 22(1), https://doi.org/10.1186/s12864-021-07464-x

Björnsson, B., Steinarsson, A., Árnason, T., 2007a. Growth model for Atlantic cod (*Gadus morhua*): Effects of temperature and body weight on growth rate. Aquaculture. 271(1–4), 216–226. https://doi.org/10.1016/j.aquaculture.2007.06.026

Björnsson, H., Olason, E. O., Jónsson, T., Henriksen, S., 2007b. Analysis of a smooth seasonal cycle with daily resolution and degree day maps for Iceland. Meteorol. Z. 16(1), 57–69. https://doi.org/10.1127/0941-2948/2007/0188

Breau, C., Cunjak, R. A., Peake, S. J., 2011. Behaviour during elevated water temperatures: Can physiology explain movement of juvenile Atlantic salmon to cool water? J. Anim. Ecol. 80(4), 844–853. https://doi.org/10.1111/j.1365-2656.2011.01828.x

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., … Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science. 359(6371), eaam7240. https://doi.org/10.1126/science.aam7240

Brett, J. R., 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerkd*). Am. Zool. 11(1), 99–113. https://doi.org/10.1093/icb/11.1.99

Burke, H., Gardner, I., Farrell, A. P., 2020. *A Review of the 2019 Newfoundland and Labrador South Coast Cultured Atlantic Salmon Mortality Event. Department of Fisheries and Land Resources, Government of Newfoundland and Labrador, Special, Studies, and Reports*. Available online at: https://www.gov.nl. ca/ffa/files/publications-pdf-2019-salmon-review-final-report.pdf (accessed March 15, 2023)

Burt, K., Hamoutene, D., Mabrouk, G., Lang, C., Puestow, T., Drover, D., Losier, R., Page, F., 2012. Environmental conditions and occurrence of hypoxia within production cages of Atlantic salmon on the south coast of Newfoundland: Occurrence of hypoxia at salmon aquaculture sites. Aquac. Res. 43(4), 607–620. https://doi.org/10.1111/j.1365-2109.2011.02867.x

CBC News, Newfoundland, 2020, March 3. Harsh weather linked to die-off at Newfoundland salmon farm. Retrieved September 25, 2021, from: https://www.cbc.ca/news/canada/newfoundland-labrador/another-salmon-die-off-1.5483364

Chen, Z., Farrell, A. P., Matala, A., Hoffman, N., Narum, S. R., 2018. Physiological and genomic signatures of evolutionary thermal adaptation in redband trout from extreme climates. Evol. Appl. 11(9), 1686-1699. https://doi.org/10.1111/eva.12672

Ćirić, J., 2020. 500 Tonnes of Salmon Die in Arnarlax Fish Farms. Icel. Rev. URL https://www.icelandreview.com/business/500-tonnes-of-salmon-die-in-arnarlax-fish-farms/ (accessed 2.21.21).

Claireaux, G., Webber, D. M., Lagardère, J.-P., & Kerr, S. R. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (Gadus morhua). J. Sea. Res. 44(3), 257–265. https://doi.org/10.1016/S1385-1101(00)00053-8

Claireaux, G., McKenzie, D. J., Genge, A. G., Chatelier, A., Aubin, J., Farrell, A. P., 2005. Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. J. Exp. Biol. 208(10), 1775–1784. https://doi.org/10.1242/jeb.01587

Corey, E., Linnansaari, T., Cunjak, R. A., Currie, S., 2017. Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo salar*). Conserv. Physiol. 5(1). https://doi.org/10.1093/conphys/cox014

Costa, I. A. S. F., Driedzic, W. R., Gamperl, A. K., 2013. Metabolic and cardiac responses of cunner *Tautogolabrus adspersus* to seasonal and acute changes in temperature. Physiol. Biochem. Zool. 86(2), 233–244. https://doi.org/10.1086/669538

Costa, I. A. S. F., Hein, T. W., Gamperl, A. K., 2015. Cold-acclimation leads to differential regulation of the steelhead trout (*Oncorhynchus mykiss*) coronary microcirculation. Am. J. Phys-Reg I., 308(9), R743–R754. https://doi.org/10.1152/ajpregu.00353.2014

Devlin, R. H., Leggatt, R. A., Benfey, T. J., 2020. Genetic Modification of Growth in Fish Species Used in Aquaculture: Phenotypic and Physiological Responses. In: Benfey, T. J., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 38, pp. 237-272. Elsevier. https://doi.org/10.1016/bs.fp.2020.09.004

Donaldson, M. R., Cooke, S. J., Patterson, D. A., Macdonald, J. S., 2008. Cold shock and fish. J. Fish. Biol. 73(7), 1491–1530. https://doi.org/10.1111/j.1095-8649.2008.02061.x

Driedzic, W. R., Bailey, J. R., Sephton, D. H., 1996. Cardiac adaptations to low temperature in non-polar teleost fish. J. Exp. Biol. 275, 186–195.

Edwards, R. (2020). Farmed salmon deaths from disease reach record high. Retrieved October 28, 2022 from:https://theferret.scot/farmed-salmon-deaths-disease-reach-record-high/#:~:text=The%20amount%20of%20salmon%20killed,than%20in%20any%20previous%20year.

Ekström, A., Gräns, A., Sandblom, E., 2019. Can´t beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. J. Comp. Physiol. B. 189(6), 757–769. https://doi.org/10.1007/s00360-019-01243-7

Eliason, E. J., Anttila, K., 2017. Temperature and the Cardiovascular System. In: Gamperl, A. K., Gillis, T. E., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 36, pp. 235–297. Elsevier. https://doi.org/10.1016/bs.fp.2017.09.003

Evans, O. (2019). Cooke loses salmon to superchilled water. Retrieved October 28, 2022 from https://salmonbusiness.com/cooke-loses-salmon-to-superchilled-water/#:~:text=Cooke%20Aquaculture%20reports%20mortalities%20at,so%2Dcalled%20%E2%80%9Csuperchill%E2%80%9D.&text=The%20company%20is%20following%20its%20Farm%20Management%20plan.

Falconer, L., Hjøllo, S. S., Telfer, T. C., McAdam, B. J., Hermansen, Ø., Ytteborg, E., 2020. The importance of calibrating climate change projections to local conditions at aquaculture sites. Aquaculture. 514, 734487. https://doi.org/10.1016/j.aquaculture.2019.734487

Farrell, A. P., Smith, F., 2017. 4—Cardiac Form, Function and Physiology. In: Gamperl, A. K., Gillis, T. E., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 36, pp. 155–264. Academic Press. https://doi.org/10.1016/bs.fp.2017.07.001

Farrell, A. P., Steffensen, J. F., (Eds.) 2005. The Physiology of Polar Fishes. Elsevier Acad. Press, Amsterdam and Heidelberg.

Farrell, A. P., Eliason, E. J., Sandblom, E., Clark, T. D., 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. 87(10), 835–851. https://doi.org/10.1139/Z09-092

Farrell, A. P., Altimiras, J., Franklin, C. E., Axelsson, M., 2013. Niche expansion of the shorthorn sculpin (*Myoxocephalus scorpius*) to Arctic waters is supported by a thermal independence of cardiac performance at low temperature. Can. J. Zool. 91(8), 573–580. https://doi.org/10.1139/cjz-2013-0038

Franklin, C. E., Farrell, A. P., Altimiras, J., Axelsson, M., 2013. Thermal dependence of cardiac function in arctic fish: Implications of a warming world. J. Exp. Biol. 216(22), 4251–4255. https://doi.org/10.1242/jeb.087130

Frölicher, T. L., Fischer, E. M., Gruber, N., 2018. Marine heatwaves under global warming. Nature. 560(7718), 360–364. https://doi.org/10.1038/s41586-018-0383-9

Gallant, M. J., LeBlanc, S., MacCormack, T. J., Currie, S., 2017. Physiological responses to a short-term, environmentally realistic, acute heat stress in Atlantic salmon, *Salmo salar*. FACETS. 2(1), 330–341. https://doi.org/10.1139/facets-2016-0053

Gamperl, A. K., Ajiboye, O. O., Zanuzzo, F. S., Sandrelli, R. M., Peroni, E. de F. C., Beemelmanns, A., 2020. The impacts of increasing temperature and moderate hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic Salmon (*Salmo salar*). Aquaculture. 519, 734874. https://doi.org/10.1016/j.aquaculture.2019.734874

Gamperl, A. K., Zrini, Z. A., Sandrelli, R. M., 2021. Atlantic Salmon (*Salmo salar*) cage-site distribution, behavior, and physiology during a Newfoundland heat wave. Front. Physiol. 12, 719594. https://doi.org/10.3389/fphys.2021.719594

Gerber, L., Clow, K. A., Gamperl, A. K., 2020a. Acclimation to warm temperatures has important implications for mitochondrial function in Atlantic salmon (*Salmo salar*). J. Exp. Biol. 224, jeb.236257. https://doi.org/10.1242/jeb.236257

Gerber, L., Clow, K. A., Mark, F. C., Gamperl, A. K., 2020b. Improved mitochondrial function in salmon (*Salmo salar*) following high temperature acclimation suggests that there are cracks in the proverbial 'ceiling.' Sci. Rep. 10(1), 21636. https://doi.org/10.1038/s41598-020-78519-4

Gollock, M. J., Currie, S., Petersen, L. H., Gamperl, A. K., 2006. Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. J. Exp. Biol. 209(15), 2961–2970. https://doi.org/10.1242/jeb.02319

Government of Newfoundland and Labrador, Canada, 2018. Climate Data: Temperature Projections (excel spreadsheet). Retrieve on October 28, 2022 from: https://www.gov.nl.ca/ecc/occ/climate-data/

Harley, C. D. G., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems: Climate change in coastal marine systems. Ecol. Lett. 9(2), 228–241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Holm, M., Holst, J. C., Hansen, L. P., 2000. Spatial and temporal distribution of post-smolts of Atlantic salmon (*Salmo salar L.*) in the Norwegian Sea and adjacent areas. ICES. J. Mar. Sci. 57(4), 955–964. https://doi.org/10.1006/jmsc.2000.0700

Huffman, J., 2019, March 17. Cooke experiences 'super chill' event off Nova Scotia coast. Retrieved September 25, 2021, from Atlantic Salmon Federation: https://www.asf.ca/news-and-magazine/salmon-news/cooke-experiences-super-chill-event-off-nova-scotia-coast

Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2017. The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. J. Exp. Biol. 220(15), 2757–2764. https://doi.org/10.1242/jeb.154021

Ibieta, P., Tapia, V., Venegas, C., Hausdorf, M., Takle, H., 2011. Chilean Salmon Farming on the Horizon of Sustainability: Review of the Development of a Highly Intensive Production, the ISA Crisis and Implemented Actions to Reconstruct a More Sustainable Aquaculture Industry. In B. Sladonja (Eds.), Aquaculture and the Environment—A Shared Destiny. InTech. https://doi.org/10.5772/30678

Iftikar, F. I., Hickey, A. J. R., 2013. Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in *Notolabrus celidotus*. Plos. One. 8(5), e64120. https://doi.org/10.1371/journal.pone.0064120

Ignatz, E. H., Braden, L. M., Benfey, T. J., Caballero-Solares, A., Hori, T. S., Runighan, C. D., Fast, M. D., Westcott, J. D., Rise, M. L., 2020. Impact of rearing temperature on the innate antiviral immune response of growth hormone transgenic female triploid Atlantic salmon (*Salmo salar*). Fish. Shellfish. Imun. 97, 656-668. https://doi.org/10.1016/j.fsi.2019.12.081

IPCC, 2018: Summary for Policymakers. In: *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (Eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 3-24, https://doi.org/10.1017/9781009157940.001.

IPCC, 2022: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., https://doi.org/10.1017/9781009325844.

Iversen, A., Asche, F., Hermansen, Ø., Nystøyl, R. 2020. Production cost and competitiveness in major salmon farming countries 2003–2018. Aquaculture. 522, 735089. https://doi.org/10.1016/j.aquaculture.2020.735089

Jacobsen, J. A., Hansen, L. P., Bakkestuen, V., Halvorsen, R., Reddin, D. G., White, J., Ó Maoiléidigh, N., Russell, I. C., Potter, E. C. E. (Ted), Fowler, M., Smith, G. W., Mork, K. A., Isaksson, A., Oskarsson, S., Karlsson, L., Pedersen, S., 2012. Distribution by origin and sea age of Atlantic salmon (*Salmo salar*) in the sea around the Faroe Islands based on analysis of historical tag recoveries. ICES. J. Mar. Sci. 69(9), 1598–1608. https://doi.org/10.1093/icesjms/fss115

Jacobsen, J., Hansen, L. P., 2001. Feeding habits of wild and escaped farmed Atlantic salmon, *Salmo salar L.*, in the Northeast Atlantic. ICES. J. Mar. Sci. 58(4), 916–933. https://doi.org/10.1006/jmsc.2001.1084

Johnson, N. C., Xie, S.-P., Kosaka, Y., Li, X., 2018. Increasing occurrence of cold and warm extremes during the recent global warming slowdown. Nat. Commun. 9(1), 1724. https://doi.org/10.1038/s41467-018-04040-y

Johnson, A. C., Turko, A. J., Klaiman, J. M., Johnston, E. F., Gillis, T. E., 2014. Cold acclimation alters the connective tissue content of the zebrafish (*Danio rerio*) heart. J. Exp. Biol. 217(11), 1868–1875. https://doi.org/10.1242/jeb.101196

Jonsson, B., Jonsson, N., 2006. Cultured Atlantic salmon in nature: A review of their ecology and interaction with wild fish. ICES. J. Mar. Sci. 63(7), 1162–1181. https://doi.org/10.1016/j.icesjms.2006.03.004

Jutfelt, F., 2020. Metabolic adaptation to warm water in fish. Funct. Ecol. 34(6), 1138–1141. https://doi.org/10.1111/1365-2435.13558

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J. R., Brijs, J., Speers-Roesch, B., Roche, D. G., Gamperl, A. K., Raby, G. D., Morgan, R., Esbaugh, A. J., Gräns, A., Axelsson, M., … Clark, T. D., 2018. Oxygen- and capacity-limited thermal tolerance: Blurring ecology and physiology. J. Exp. Biol. 221(1), jeb169615. https://doi.org/ 10.1242/jeb.169615

Keen, A. N., Gamperl, A. K., 2012. Blood oxygenation and cardiorespiratory function in steelhead trout (*Oncorhynchus mykiss*) challenged with an acute temperature increase and zatebradine-induced bradycardia. J. Therm. Biol. 37(3), 201–210. https://doi.org/10.1016/j.jtherbio.2012.01.002

Keen, A. N., Klaiman, J. M., Shiels, H. A., Gillis, T. E., 2017. Temperature-induced cardiac remodelling in fish. J. Exp. Biol. 220(2), 147–160. https://doi.org/10.1242/jeb.128496

Klaiman, J. M., Pyle, W. G., Gillis, T. E., 2014. Cold acclimation increases cardiac myofilament function and ventricular pressure generation in trout. J. Exp. Biol. 217, 4132-4140. https://doi.org/10.1242/jeb.109041

Lacroix, G. L., Knox, D., 2005. Distribution of Atlantic salmon (*Salmo salar*) postsmolts of different origins in the Bay of Fundy and Gulf of Maine and evaluation of factors affecting migration, growth, and survival. Can. J. Fish. Aquat. Sci. 62, 14. https://doi.org/10.1139/F05-055

Lhorente, J. P., Araneda, M., Neira, R., Yáñez, J. M., 2019. Advances in genetic improvement for salmon and trout aquaculture: the Chilean situation and prospect. Rev. Aquacult. 11(2), 340-353. https://doi.org/10.1111/raq.12335

Liu, C., Ge, J., Zhou, Y., Thirumurugan, R., Gao, Q., Dong, S., 2020. Effects of decreasing temperature on phospholipid fatty acid composition of different tissues and hematology in Atlantic salmon (*Salmo salar*). Aquaculture. 515, 734587. https://doi.org/10.1016/j.aquaculture.2019.734587

Lurman, G. J., Petersen, L. H., Gamperl, A. K., 2012. In situ cardiac performance of Atlantic cod (*Gadus morhua*) at cold temperatures: Long-term acclimation, acute thermal challenge and the role of adrenaline. J. Exp. Biol. 215(22), 4006–4014. https://doi.org/10.1242/jeb.069849

Moore, A., Bendall, B., Barry, J., Waring, C., Crooks, N., Crooks, L., 2012. River temperature and adult anadromous Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*: Temperature and Salmonids. Fish. Manag. Ecol. 19(6), 518–526. https://doi.org/10.1111/j.1365-2400.2011.00833.x

Motyka, R., Norin, T., Petersen, L. H., Huggett, D. B., Gamperl, A. K., 2017. Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss)*, but does not affect their upper thermal tolerance. J. Therm. Biol. 68, 149–161. https://doi.org/ 10.1016/j.jtherbio.2016.03.007

Norin, T., Canada, P., Bailey, J. A., Gamperl, A. K., 2019. Thermal biology and swimming performance of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). PeerJ. 7, e7784. https://doi.org/10.7717/peerj.7784

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., Wernberg, T., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9(1), 1324. https://doi.org/10.1038/s41467-018-03732-9

Olsvik, P. A., Vikeså, V., Lie, K. K., Hevrøy, E. M., 2013. Transcriptional responses to temperature and low oxygen stress in Atlantic salmon studied with next-generation sequencing technology. BMC. Genomics. 14(1), 817. https://doi.org:10.1186/1471-2164-14-817

Penney, C. M., Nash, G. W., Gamperl, A. K., 2014. Cardiorespiratory responses of seawater-acclimated adult Arctic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) to an acute temperature increase. Can. J. Fish. Aquat. Sci. 71(7), 1096–1105. https://doi.org/10.1139/cjfas-2013-0569

Pörtner, H. O., 2010. Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213(6), 881–893. https://doi.org/10.1242/jeb.037523

Pörtner, H. O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science. 315(5808), 95–97. https://doi.org/10.1126/science.1135471

Reid, G., Gurney-Smith, H., Flaherty, M., Garber, A., Forster, I., Brewer-Dalton, K., Knowler, D., Marcogliese, D., Chopin, T., Moccia, R., Smith, C., De Silva, S., 2019. Climate change and aquaculture: Considering adaptation potential. Aquacult. Env. Intera. 11, 603–624. https://doi.org/10.3354/aei00333

Riseth, E. N., Fraser, T. W. K., Sambraus, F., Stien, L. H., Hvas, M., 2020. Is it advantageous for Atlantic salmon to be triploid at lower temperatures? J. Therm. Biol. 89, 102548. https://doi.org/10.1016/j.jtherbio.2020.102548

Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., Odelström, A., Adill, A., Aho, T., Jutfelt, F., 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nat. Commun. 7(1), 11447. https://doi.org/10.1038/ncomms11447

Sandnes, K., Lie, O., Waagbo, R., 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish. Biol. 32(1), 129-136. https://doi.org/10.1111/j.1095-8649.1988.tb05341.x

Saunders, R. L., Muise, B. C., Henderson, E. B., 1975. Mortality of salmonids cultured at low temperature in sea water. Aquaculture. 5(3), 243–252. https://doi.org/10.1016/0044-8486(75)90002-2

Saunders R. L., Fletcher, G. L., Hew, C. L., 1998. Smolt development in growth hormone transgenic Atlantic salmon. Aquaculture. 169(1-4), 177-193. https://doi.org/10.1016/S0044-8486(98)00348-2

Seafood Industry Year in Review (SIYIR), 2020. Aquaculture: Salmonids. Retrieved October 28, 2022 from: https://www.gov.nl.ca/ffa/files/2020-SIYIR-WEB.pdf

Stehfest, K. M., Carter, C. G., McAllister, J. D., Ross, J. D., Semmens, J. M., 2017. Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors. Sci. Reps. 7(1), 4545. https://doi.org/10.1038/s41598-017-04806-2

Szekeres, P., Eliason, E., Lapointe, D., Donaldson, M., Brownscombe, J., Cooke, S. 2016. On the neglected cold side of climate change and what it means to fish. Climate. Res. 69(3), 239–245. https://doi.org/10.3354/cr01404

Strøm, J. F., Thorstad, E. B., Rikardsen, A. H., 2020. Thermal habitat of adult Atlantic salmon *Salmo salar* in a warming ocean. J. Fish. Biol. 96(2), 327–336. https://doi.org/10.1111/jfb.14187

Taugbøl, A., Olstad, K., Bærum, K. M., Museth, J., 2019. Swimming performance of brown trout and grayling show species-specific responses to changes in temperature. Ecol. Freshw. Fish. 28(2), 241–246. https://doi.org/10.1111/eff.12447

The Canadian Press. (2015, March 12). Extreme cold cause of fish deaths at N.S. aquaculture sites. Retrieved September 25, 2021 from CTV News: https://atlantic.ctvnews.ca/extreme-cold-cause-of-fish-deaths-at-n-s-aquaculture-sites-1.2276322

The North Atlantic Salmon Conservation Organization (NASCO), 2021. Compilation of NASCO Catch Statistics – 2021 (excel spreadsheet). Retrieved October 28, 2022, from: https://nasco.int/ices-2/statistics/

Tromp, J. J., Jones, P. L., Brown, M. S., Donald, J. A., Biro, P. A., Afonso, L. O. B., 2018. Chronic exposure to increased water temperature reveals few impacts on stress physiology and growth responses in juvenile Atlantic salmon. Aquaculture. 495, 196–204. https://doi.org/10.1016/j.aquaculture.2018.05.042

Tveteras, R., Nystoyl, R., Jory, D. E., 2019. GOAL 2019: Global finfish production review and forecast. Retrieved October 28, 2022 from: https://www.aquaculturealliance.org/advocate/goal-2019-global-finfish-production-review-and-forecast/#:~:text=In%202018%2C%20production%20increased%20by,13%20million%20MT%20in%202019.

Vadboncoeur, E., Nelson, C., Hall, J.R., Clow, K.A., Sandrelli, R.M., Brauner, C.J., Swanson, A.K., Gamperl, A.K., 2023. Lowering temperature to 1˚C results in physiological changes, stress and mortality in cultured Atlantic salmon (*Salmo salar*). Aquaculture. https://doi.org/10.1016/j.aquaculture.2023.739313

Wade, N. M., Clark, T. D., Maynard, B. T., Atherton, S., Wilkinson, R. J., Smullen, R. P., Taylor, R. S., 2019. Effects of an unprecedented summer heatwave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (*Salmo salar*). J. Therm. Biol. 80, 64-74. https://doi.org/10.1016/j.jtherbio.2018.12.021

Wang, T., Overgaard, J., 2007. Ecology: The heartbreak of adapting to global warming. Science. 315(5808), 49–50. https://doi.org/10.1126/science.1137359

Wang, C., Liu, H., Lee, S-K., 2010. The record-breaking cold temperatures during the winter of 2009/2010 in the Northern Hemisphere. Atmos. Sci. Lett. 11(3), 161-168. https://doi.org/10.1002/asl.278

Weitzman, J., Bailey, M., 2019. Communicating a risk-controversy: Exploring the public discourse on net-pen aquaculture within the Canadian media. Aquaculture. 507, 172–182. https://doi.org/10.1016/j.aquaculture.2019.04.025

CHAPTER 2: Acute and Chronic Cold Exposure Differentially Affect Cardiac Control, but Not Cardiorespiratory Function, in Resting Atlantic Salmon (*Salmo salar*)

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Abstract

No studies have examined the effects of cold temperatures (~ 0-1°C) on *in vivo* cardiac function and control, and metabolism, in salmonids. Thus, we examined: 1) how acclimation to 8°C vs. acclimation (>3 weeks) or acute exposure (8-1°C at 1°C h^{-1}) to 1°C influenced cardiorespiratory parameters in resting Atlantic salmon; and 2) if/how the control of cardiac function was affected. Oxygen consumption ($\dot{M}O_2$) and cardiac function [i.e., heart rate (f_H) and cardiac output ($\dot{Q}$)] were 50% lower in the acutely cooled and 1°C-acclimated salmon as compared to 8°C fish, whereas stroke volume (V_S) was unchanged. Intrinsic f_H was not affected by whether the fish were acutely exposed or acclimated to 1°C (values ~51, 24 and 21 beats min^{-1} in 8 and 1°C-acclimated fish, and 8-1°C fish, respectively), and in all groups f_H was primarily under adrenergic control/tone (cholinergic tone 13–18%; adrenergic tone 37-70%). However, β-adrenergic blockade resulted in a 50% increase in V_S in the 1°C-acclimated group, and this was surprising as circulating catecholamine levels were ~1-3 nM in all groups. Overall, the data suggest that this species has a limited capacity to acclimate to temperatures approaching 0°C. However, we cannot exclude the possibility that cardiac and metabolic responses are evoked when salmon are cooled to ~ 0-1°C, and that this prevented further declines in these parameters (i.e., they 'reset' quickly). Our data also provide further evidence that V_S is temperature insensitive, and strongly suggest that changes in β-adrenoreceptor mediated control of venous pressure/capacitance occur when salmon are acclimated to 1°C.

2.1 Introduction

Climate change is predicted to increase average ocean temperatures by approximately 2 to 4°C by the year 2100 (IPCC, 2021), and is increasing the severity and frequency of harmful conditions/events: including storms, marine heat waves, extreme reductions in water temperatures (i.e., 'cold shock' events) and hypoxia (Breitburg et al., 2018; Frölicher et al., 2018; Johnson et al., 2018; Oliver et al., 2018; Sampaio et al. 2021; Szekeres et al., 2016). Numerous biological functions in aquatic ectotherms, including fish, are affected by temperature as it is the 'ecological master factor' (Brett, 1971). Changes in physiological processes due to increased temperatures have been the main focus of climate change-related research in fish, and have involved measurements of upper thermal tolerance [critical thermal (CT_{Max}) and incremental temperature maxima (IT_{Max}): Gamperl et al., 2020; Gallant et al., 2017; Leeuwis et al., 2019; Zanuzzo et al., 2019], and cardiorespiratory parameters. These parameters include oxygen consumption ($\dot{M}O_2$), cardiac output (the amount of blood pumped by the heart in 1 minute; $\dot{Q}$), heart rate (f_H), stroke volume (the amount of blood pump in a heart beat; V_S), blood oxygen transport and tissue oxygen extraction (Anttila et al., 2014; Ekström et al., 2019; Gollock et al., 2006; Hvas et al., 2017; Leeuwis et al., 2021; Motyka et al., 2017; Penney et al., 2014) given their purported importance to thermal tolerance (Farrell et al., 2009; Jutfelt et al., 2018; Pörtner, 2010; Wang and Overgaard, 2007). However, with the exception of Antarctic species (Davison et al., 1997; Farrell and Steffensen, 2005), and a few other 'polar' species (Drost et al., 2016; Farrell et al., 2013; Franklin et al., 2013), there is very limited literature describing the effects of low (< 5°C) or cold (~0-1°C) temperatures on fish *in vivo* cardiovascular physiology. Costa et al. (2013) examined the effect of an acute temperature decrease from 8 to 1°C, and acclimation to these temperatures, on *in vivo* cardiorespiratory parameters in the cunner (*Tautogolabrus adspersus*). However, this species is

capable of metabolic depression at low temperatures (Costa et al., 2013; Gerber et al., in final prep), and thus, the results of this research are unlikely to be representative of most teleosts. Data on temperature dependent changes collected using *in situ* or *in vitro* preparations are limited (e.g., Aho and Vornanen, 2001; Gamperl and Syme, 2021; Lurman et al., 2012), and may not accurately reflect temperature-dependent responses in *in vivo* cardiac performance. Finally, other literature on this topic is largely restricted to: the investigation of cardiac remodelling (including changes in relative ventricular mass; RVM) (Aho and Vornanen, 2001; Driedzic et al., 1996; Eliason and Anttila, 2017; Johnson et al., 2014; Keen et al., 2017; Klaiman et al., 2011); or to experiments where anesthetized fish were given pharmacological agents (atropine and isoproterenol) and exposed to very rapid acute changes in temperature so that their 'maximum' heart rate and its Arrhenius breakpoint at high temperatures could be measured (e.g., see Gilbert et al, 2020; Gilbert and Farrell, 2021)

Increased levels of stress biomarkers (i.e., plasma concentrations of cortisol, adrenaline (AD) and noradrenaline (NAD), and of tissue heat shock proteins) are strongly linked to stress exposure, including thermal stress (Alzaid et al., 2015; LeBlanc et al., 2012; Leeuwis et al., 2021; Sandblom and Axelsson, 2011). The release of catecholamines (primarily AD and NAD) from the chromaffin cells of the head kidney is a central component of the stress response (Reid et al., 1998), and modulates cardiac performance in fish (Farrell and Smith, 2017). However, there is also adrenergic nervous tone on the heart which varies between species, and is critical for supporting cardiac function when fish are exposed to stressors, including changes in temperature (Sandblom and Axelsson, 2011). Pharmacological blockade can be used to determine the contribution of parasympathetic and sympathetic influences (Altimiras et al., 1997; Axelsson, 1988; Butler et al., 1989; Farrell et al., 1986) on f_H and $\dot{Q}$, and to determine a fish's intrinsic heart

rate [i.e., the frequency of action potentials generated by the pacemaker cells in the sinoatrial node (SAN)] when other influences are removed (Aho and Vornanen, 2001; Ekström et al., 2016a; Mendonca and Gamperl, 2009; Sandblom and Axelsson, 2011)]. Such research is important in the context of climate change as the time course for the resetting of cardiac frequency (f_H) following acute (within hours) or prolonged / chronic (days to months) changes with temperature are not well understood, and the data are contradictory/variable. For example, the time required to reset the f_H in rainbow trout was described by Sutcliff et al. (2020) to be just 1 hour in fish acclimated to 4°C in winter and exposed to 12°C, and 8 hours for fish acclimated to 12°C and transferred to 4°C. However, these authors did not see such an effect even after 10 weeks of acclimation to these temperatures in summer-acclimated fish. In addition, other studies have concluded that the resetting of f_H requires several weeks in fishes (Aho and Vornanen, 2001; Ekström et al., 2016a).

Cold temperatures are a significant challenge to the Atlantic salmon aquaculture industry and can impact fish welfare and production, especially in countries such as Canada and Iceland. Temperatures normally range seasonally and with depth at sea-cages from ~ 2 to 20°C (Björnsson et al., 2007a and b; Gamperl et al., 2021; Gollock, 2006). However, chronic exposure to cold (~ 0-1°C) temperatures during the winter months, as well as acute daily drops (to < 2°C) due to 'turn over' caused by rough conditions, are associated with large-scale mortalities at salmon sea-cages in these countries (Círíc, 2020; CBC News, 2020; Huffman, 2019; The Canadian Press, 2015). Our understanding of how warm (summer) temperatures affect cultured Atlantic salmon has improved greatly over the past few years (Anttila et al., 2014; Beemelmanns et al., 2021ab; Gamperl et al., 2020; Gamperl and Syme, 2021; Hvas et al., 2017; Penney et al., 2014; Tromp et al., 2018). However, we have a very limited understanding of how the biology and physiology of this species is affected by short-term and long-term exposure to cold temperatures, and the results

of this research can be contradictory (e.g., see Liu et al., 2020 vs. Sandnes et al., 1988). As part of a larger, comprehensive, research program on how cultured Atlantic salmon are impacted by cold (~0-1°C) temperatures (including ionoregulation, cage-site behaviour and physiology, levels of stress biomarkers, tissue damage etc.), this study examined how acute exposure (i.e., a temperature drop over 8 hours) and chronic acclimation to 1°C: 1) influenced the salmon's resting cardiorespiratory function (f_H, V_S, $\dot{Q}$, and $\dot{M}O_2$); 2) affected blood O_2-carrying capacity [haematocrit and haemoglobin] and circulating stress hormone levels; and 3) affected cholinergic and adrenergic tones on the heart, and intrinsic f_H, in this species.

2.2 Materials and Methods

This study was approved by the Animal Care Committee of Memorial University of Newfoundland and Labrador (protocol #21-01-KG). All procedures conducted on the salmon were performed in accordance with the Canadian Council on Animal Care's Guidelines on the 'Care and Use of Fish in Research, Teaching and Testing' (Canadian Council on Animal Care, 2005).

2.2.1 Fish husbandry and rearing conditions

Male Atlantic salmon were held in 0.8 m^3 tanks in the Annex Tank Room of the Ocean Science Center at Memorial University. The tanks were initially supplied with seawater (~ 32 ppt) with temperature and oxygen levels maintained at 8°C and >95% of air saturation respectively, and with a 12 h light: 12 h dark photoperiod. There were two tanks each with 25 fish, and after approx. 2-3 weeks in the tanks, one tank remained at 8°C, whereas the other tank had its temperature decreased from 8°C to 1°C at 1°C per week (i.e., over 7 weeks). After all fish were acclimated to their respective temperatures for > 3 weeks (range 3 to 8 weeks), the fish were used

in experiments to examine the effect of acclimation to 1°C, and an acute (over 7 hours) drop in temperature from 8°C to 1°C, on Atlantic salmon cardiorespiratory function and stress physiology (see below). A custom-built chiller made by Technical Services at Memorial University was used to provide water at 1°C. This was the lowest temperature used as it was difficult to consistently achieve a temperature of 0°C with this system / avoid temperatures going below 0°C. All fish were fed a commercial salmon diet (5 mm, EWOS, Canada) by hand at ~1% body mass three times per week (i.e., a maintenance diet) and fish were not fed within 24 h of surgery. The weight [1.1 ± 0.7 kg; mean ± standard error (s.e.m.)], fork length (42.3 ± 0.9 cm), condition factor (1.47 ± 0.01), and relative ventricular mass (0.745 ± 0.003%) were similar for all fish used in the experiment (see Supplementary material, Table S1).

2.2.2 Surgical procedures and recovery

Fish were netted from their tank, and anaesthetized in oxygenated seawater containing tricaine methanesulfonate (MS-222, 0.2 g L^{-1}; Syndel Laboratories Ltd., Qualicum Beach, BC, Canada) until ventilatory movements ceased. The fish were then weighed and measured for fork length, and placed supine on a wetted foam pad upon a surgical table where their gills were irrigated continuously with cooled (~ 4°C) and oxygenated seawater containing a maintenance dose of MS-222 (0.1 g L^{-1}). Each fish was fitted with a dorsal aortic cannula (PE 50, Clay-Adams; Becton Dickensen and Co., Sparks, MD, USA) as in Smith and Bell (1964) and Gamperl et al. (1994a) to allow for blood collection and for the injection of antagonists of cardiac control (see below). Following cannulation, the salmon were placed on their right side, and a Doppler® flow probe (Model ES Cuff-type Transducer, 20 MHz, Iowa Doppler Products, Iowa City, IA, USA) ranging from 1.3 to 2 mm in diameter (depending on fish size) was fitted around the ventral aorta

using the same procedures as described for rainbow trout in Gamperl et al. (1994a). Finally, the flow probe lead was connected to a directional pulsed Doppler® flow meter (Model 545C-4; Bioengineering, University of Iowa, Ames, IA, USA) to ensure that the signal was of high quality, and the probe lead was secured to the fish at 3 locations: just posterior to the pectoral fin, just below the lateral line, and just ventral to the dorsal fin.

After surgery was completed, each fish (N = 9 fish per group) was placed in a 20 L cylindrical respirometer (20 cm in diameter × 54.6 cm long) to recover for ~18 to 24 h (i.e., until the first morning prior to experiments). The respirometers were submersed in a shallow (25 cm deep), insulated, experimental water table containing fully aerated seawater at either 8°C or 1°C (the fish's acclimation temperature) and received a constant flow of water at a rate of 10 L min^{-1} from a submersible Eheim pump (model 1048; EHEIM GmbH & Co., Deizisau Germany). Water in the experimental water table was supplied from a large (~300 L) reservoir whose temperature was controlled by a custom-designed heater/chiller (Technical Services, Memorial University of Newfoundland).

2.2.3 Acute temperature decrease

The morning following surgery (~18 to 24 h post-surgery), the flow probe leads were connected to the Doppler® flow meter and the fish were left undisturbed for another ~1 h before initial measurements were taken. Then, two groups remained at their respective acclimation temperatures (8 and 1°C), while the third group was exposed to a decrease in temperature from 8°C to 1°C at 1°C h^{-1}. Cardiac function and oxygen consumption measurements were subsequently made in all groups at times corresponding to every 1°C decrease in temperature in the one group, during the second night (every 20 minutes), at 48 hours after surgery (the second morning), and

before and after the administration of antagonists of parasympathetic and sympathetic cardiac control. These pharmacological antagonists [atropine sulfate (1.2 mg kg^{-1}) and propranolol hydrochloride (3 mg kg^{-1})] were sequentially injected (separated by 90 minutes) through the cannula, followed by 0.4 mL of saline (0.9% NaCl) to clear/flush the cannula (Mendonca and Gamperl, 2009). Blood samples (1.5 mL) were withdrawn from the dorsal aortic cannula and immediately replaced with saline at three time points: 1) 24 hours post-surgery (i.e., prior to changes in temperature); 2) 1 hour after the treatment fish reach 1°C (and at the same time in the other groups); and 3) just prior to the injection of the pharmacological antagonists (i.e., approx. 48 hours post-surgery). A schematic representation of the experimental design is provided in **Figure 2-1**.

After the experiment was completed, all fish were euthanized using a lethal dose of 0.4 g L^{-1} of MS-222, and an *in situ* post-mortem calibration of each flow probe was performed at physiologically relevant pressures using a peristaltic pump (MasterFlex Easyload®, Quebec, Canada) (Gamperl et al., 1994a) and a 'blood mimicking' solution (0.99% glycerol, 2.4% TritonX, 35% Orgasol in 200 mL of distilled water; Axelsson, pers. comm.). To accomplish this, after removal of the sinus venosus and atrium, the ventricle was bisected laterally, and a steel cannula attached to peristaltic pump tubing was tied into the ventricular lumen. Finally, the two halves of the ventricle were weighed, and relative ventricular mass (RVM) was calculated as:

(1) [(ventricular mass / fish mass) x 100]

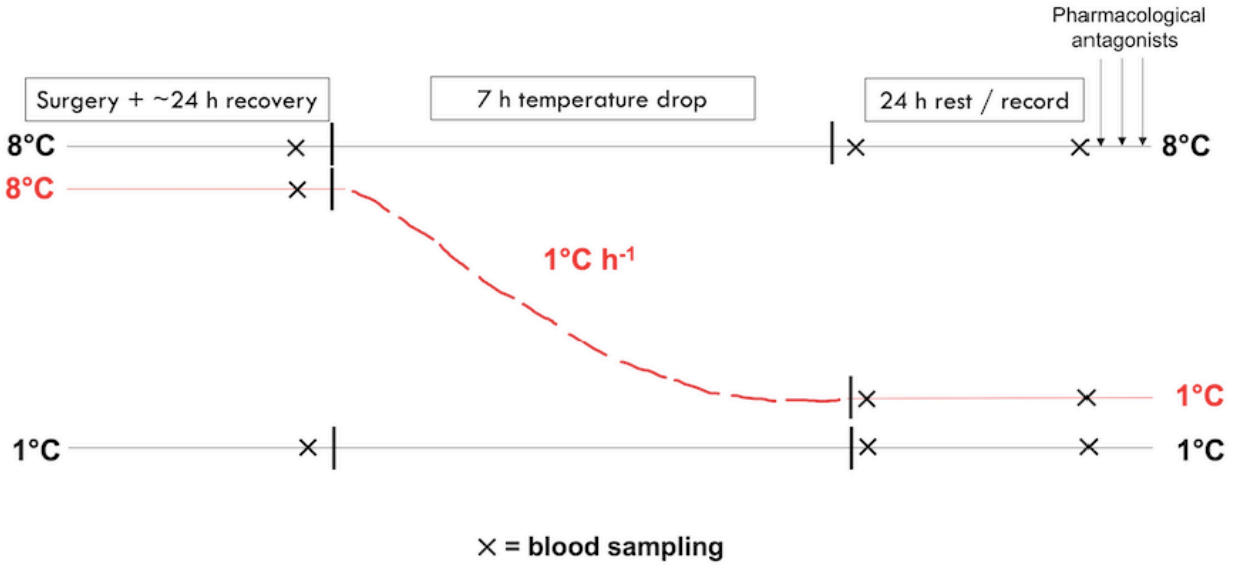

Figure 2-1. Schematic diagram depicting the experimental design used to assess the effect of cold temperatures on the cardiorespiratory and stress physiology of the 3 groups of fish: acclimated and tested at 8°C; acclimated and tested at 1°C; acclimated to 8°C and acutely exposed to a drop in temperature to 1°C. The cardiorespiratory parameters (f_H, V_S, $\dot{Q}$ and $\dot{M}O^2$) were recorded at the fish's acclimation temperature, at each 1°C decrease in temperature, at rest on the second morning, and before each of the drug injections. Blood samples for various haematological parameters were taken (as indicated by an **X**) at the fish's acclimation temperature, an hour after these fish reached 1°C, and on the morning of the 2nd day prior to drug injection (note: all groups were sampled at the same time points).

2.2.4 Cardiorespiratory function and neurohormonal control

Oxygen consumption ($\dot{M}O_2$; in mg O_2 kg^{-1} h^{-1}) was measured using automated intermittent closed respirometry (Sandblom et al., 2014) and using methods consistent with recommendations for aquatic respirometry as detailed in Killen et al. (2021), Rodgers et al. (2016) and Svendsen et al. (2016). The flush pump and recirculation pump (both Eheim Model 1048; 10 L min^{-1}) were controlled by AutoResp® software (Version 2.1.0; LoligoSystems, Tjele, Denmark), and were intermittently turned on and off to either flush the respirometer with fresh seawater when on, or create a functionally sealed respirometry chamber when off. The duration of the 'flush' and/or 'recirculation' periods were adjusted throughout the experimental period to ensure a regression coefficient (R^2) > 0.75 was achived for the decline in water O_2 level and to avoid a PO_2 inside the respirometers lower than 85% air saturation. This lower limit ensured that the water in the respirometer would return to 100% air saturation prior to the next measurement, and that the haemoglobin leaving the gills would still be near full O_2 saturation (Nikinmaa and Soivio, 1979). An R^2 > 0.75 was used due to the large signal to noise ratio exhibited by fish at colder temperatures (i.e., at 1 °C) when at rest or during measurements of SMR (see below), to ensure that MO_2 was not overestimated (Chabot et al., 2020). However, the average R^2 was > 0.9. $\dot{M}O_2$ recordings were made using an OXY-4 mini fiber-optic oxygen meter fitted with pre-calibrated dipping probes (PreSens Precision Sensing GmBH, Regensburg, Germany) that was interfaced with DAQ-4 and TEMP-4 modules (LoligoSystems, Tjele, Denmark). The signals from these modules were then fed into a computer running AutoResp® software. The rate of oxygen decline during the closed phase of the respirometry cycle (i.e., when the flush pump was off) was used by the AutoResp® software to calculate the $\dot{M}O_2$ of the fish after a 2 min 'wait' period at the beginning of the closed period (which varied between 5 and 15 minutes depending on temperature to ensure an acceptable

R^2). Given the limited number of $\dot{M}O_2$ measurements, standard metabolic rate (SMR) was determined during the 18h following day 2 (i.e., after the acute temperature decrease) by calculating the mean of the lowest 20% of the $\dot{M}O_2$ measurements after the removal of values with an $R^2 < 0.75$ (2 / 396, 66 / 392, and 33 / 332 for 8, 1 and 8 to 1 °C fish, respectively) (Chabot et al., 2016). To avoid external influences on fish respiration (Speers-Roeshe et al., 2018) the lights remained on following surgery until the end of the experiments, and the water table was surrounded by a 0.8 m high corrugated black plastic sheet to prevent the fish from being disturbed by the presence of research personnel. Background measurements of $\dot{M}O_2$ were made in empty chambers at the end of the experiments, and these were negligible (< 1%), indicating that no substantial microbial respiration was occurring (Rodgers et al., 2016; Svendsen et al., 2016).

Heart rate and cardiac output were recorded by connecting the flow probe leads to a pulsed Doppler® flow meter. Signals from the Doppler® flow meter were amplified and filtered using a data acquisition system (MP100A-CE; BIOPAC Systems, Inc., Santa Barbara, CA, USA) and a universal interface module (UIM100C; BIOPAC Systems, Inc.) connected to a laptop computer running AcqKnowledge® software (Version 3.8.2; BIOPAC Systems, Inc.). Heart rate (f_H; beats min^{-1}) was determined by measuring the number of systolic peaks during two 30s intervals during the period when $\dot{M}O_2$ was being measured. The values for $\dot{Q}$ were recorded in volts (V), and were converted to mL min^{-1} kg^{-1} using the data from the calibrations described previously and the fish's weight (in kg). Stroke volume (V_S) was calculated as $\dot{Q} / f_H$. Oxygen extraction (the amount of O_2 consumed per mL of blood pumped) was estimated by dividing $\dot{M}O_2$ by $\dot{Q}$.

Q_{10} values (i.e., the fractional change in a rate over a 10°C range) were calculated as an index of the effect of chronic (acclimation) and acute temperatures (T) changes (8 to 1°C) on $\dot{M}O_2$, f_H and $\dot{Q}$ and $MO_2 / \dot{Q}$ (R) using the following equation:

(2) $Q_{10} = \left(\frac{R2}{R1}\right)^{\left(\frac{10}{(T2-T1)}\right)}$

The 'intrinsic' f_H (f_{Hint}; beats min^{-1}) after the administration of both drugs, cholinergic tonus (%f_{Hch}; %) and adrenergic tonus (%f_{Had}; %) were calculated for the salmon's heart rate as described by Axelsson (1988):

(3) % f_{Hch} = [(f_H after atropine – f_H before atropine) / f_{Hint}] x 100

(4) % f_{Had} = [(f_H after atropine – f_{Hint}) / f_{Hint}] x 100

2.2.5 Haematological parameters

Blood samples (1.5 mL) were collected (see previously provided sampling details) and immediately aliquoted for the analysis of various haematological parameters. First, blood was drawn into microhaematocrit tubes and centrifuged at 10,000 x*g* for 5 min to determine haematocrit (Hct; %). An aliquot of 50 µL of whole blood was then collected for the measurement of blood haemoglobin (Hb) concentration using the cyanomethaemoglobin method (Drabkins reagent, D5941; Sigma Aldrich, Oakville, Canada) and the absorbance was read at 540 nm using a plate reader (SpectraMax 5, Molecular Devices, San Jose, USA). Hb concentrations were calculated from standard curves generated using bovine Hb (Sigma, H2500). Mean cellular Hb concentration (MCHC, in mg mL^{-1}) was calculated as Hb concentration / Hct × 100. The remaining blood sample was centrifuged for 1 min at 10,000 x*g* in a mini-centrifuge (05-090-128, Fisher Scientific). Three hundred µL of plasma was then pipetted into a 1.5 mL, opaque, Eppendorf® tube containing 15 µL of 0.2 M EDTA and 15 µL of 0.15 M glutathione (which served as antioxidants) for later measurement of circulating catecholamine levels. The remaining plasma was pipetted into

50 μL aliquots for the measurement of plasma cortisol and lactate. All samples were immediately frozen in liquid N_2 and stored at -80°C.

2.2.6 Measurement of plasma lactate, cortisol and catecholamines

ELISA kits were used to measure plasma levels of cortisol (Neogen Life Sciences, 402710, Lexington, KY, USA) and adrenaline and noradrenaline (Abnova KA1877, Taipei, Taiwan), following the manufacturer's instructions. Plasma samples for catecholamine measurement were analysed within 2 months of storage at -80°C. Plasma lactate was first deproteinized with 6% (v/v) perchloric acid, then measured spectrophotometrically at 340 nm using the production of NADH/NADPH by lactate dehydrogenase (Sigma L2500), and [lactate] was calculated in reference to standard curves (Sigma L6402).

2.2.7 Statistical Analyses

All statistical analyses were performed using Rstudio v. 1.4.1717 with R v. 4.1.0 (R Core Team, 2021). A Rosner's Test for outliers ($\alpha = 0.05$; which was selected as the best test for the identification of outliers by R using the *EnvStats* package) was used on all datasets prior to statistical analysis (Millard, 2013). This analysis revealed that there were very few outliers: these include the majority of cardiorespiratory measurements for one fish in the 8°C-acclimated group, and lactate data at one sampling point for one of the acutely cooled fish. A general linear model (lm function) and ANOVAs were used with the *stats* package to assess the main factor of 'group' on morphometric variables [mean weight (g), fork length (cm), condition factor (K) and RVM], SMR, intrinsic heart rate (f_{Hint}), and the various values of 'tone' on f_H between the three groups. If there was a significant effect ($p < 0.05$), a Tukey's HSD *post-hoc* test from the *stats* package

examined where the differences occurred. A general linear mixed model [lmer function in the *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages in R] was used to analyze all cardiorespiratory, pharmacologically-induced and haematological data. This model included 'fish' as a random factor, and 'group' and 'time' of measurement' and their interaction as fixed effects. Main effects were analyzed using ANOVAs (anova function in the *stats* package in R) with type III sums of squares, and if the model indicated a significant fixed effect ($p < 0.05$), a Bonferroni (fdr method in the *stats* package in R) *post-hoc* test identified statistical differences . Data for plasma lactate were transformed ($x^{-0.333}$) prior to statistical analysis as this parameter failed Shapiro-Wilks normality test (Fox and Weisberg, 2019). All data shown are means ± s.e.m., and $p < 0.05$ was used as the threshold for determining statistical significance.

2.3 Results

2.3.1 Haematological response to cold exposure

There were no differences in Hct or [Hb] between the groups. However, on average, all groups had slightly lower Hct (%) values during the third sampling as compared to the first sampling (values ~ 28 – 30%), in addition to significantly lower [Hb] during the second and third samplings (**Figure 2-2a and b**) compared to the first sampling (108.9 ± 6.0 mg mL^{-1} and 103.4 ± 5.6 mg mL^{-1} vs 122.2 ± 5.7 mg mL^{-1} respectively). No significant differences in MCHC were observed during this experiment, with values approx. 350 – 450 ng mL^{-1} (**Figure 2-2c**). Lactate decreased significantly in fish exposed to the gradual decrease in temperature from the first to second sampling (an hour after they had reached 1°C); i.e., from 0.69 ± 0.17 mM to 0.41 ± 0.15 mM, respectively (**Figure2-3d**), and the following morning it was 0.57 ± 0.17 mM. However, these values were not significantly different than those measured in the other two groups.

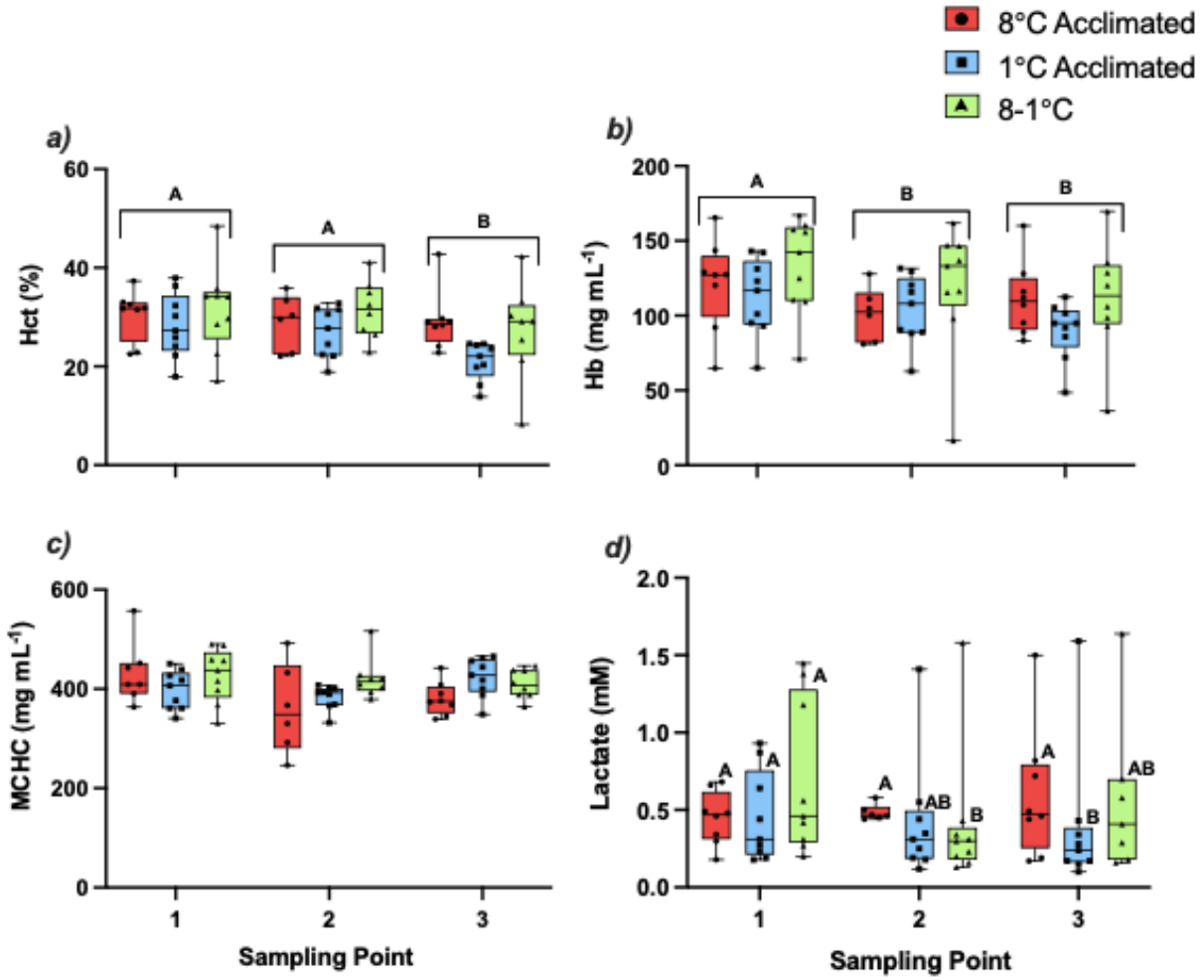

Figure 2-2. Haematological parameters in Atlantic salmon acclimated to 8 (*red*) and 1°C (*blue*), and acutely cooled from 8 to 1°C (*green*). Shown are graphs of (*a*) haematocrit (Hct), (*b*) haemoglobin (Hb), (*c*) mean cellular Hb concentration (MCHC), and (*d*) plasma lactate. Symbols without a letter in common are significantly different ($p < 0.05$) between groups at a particular sampling point (lowercase) and between the sampling points within a group (uppercase). Values are means ± 1 s.e.m. with n = 6-9 per group.

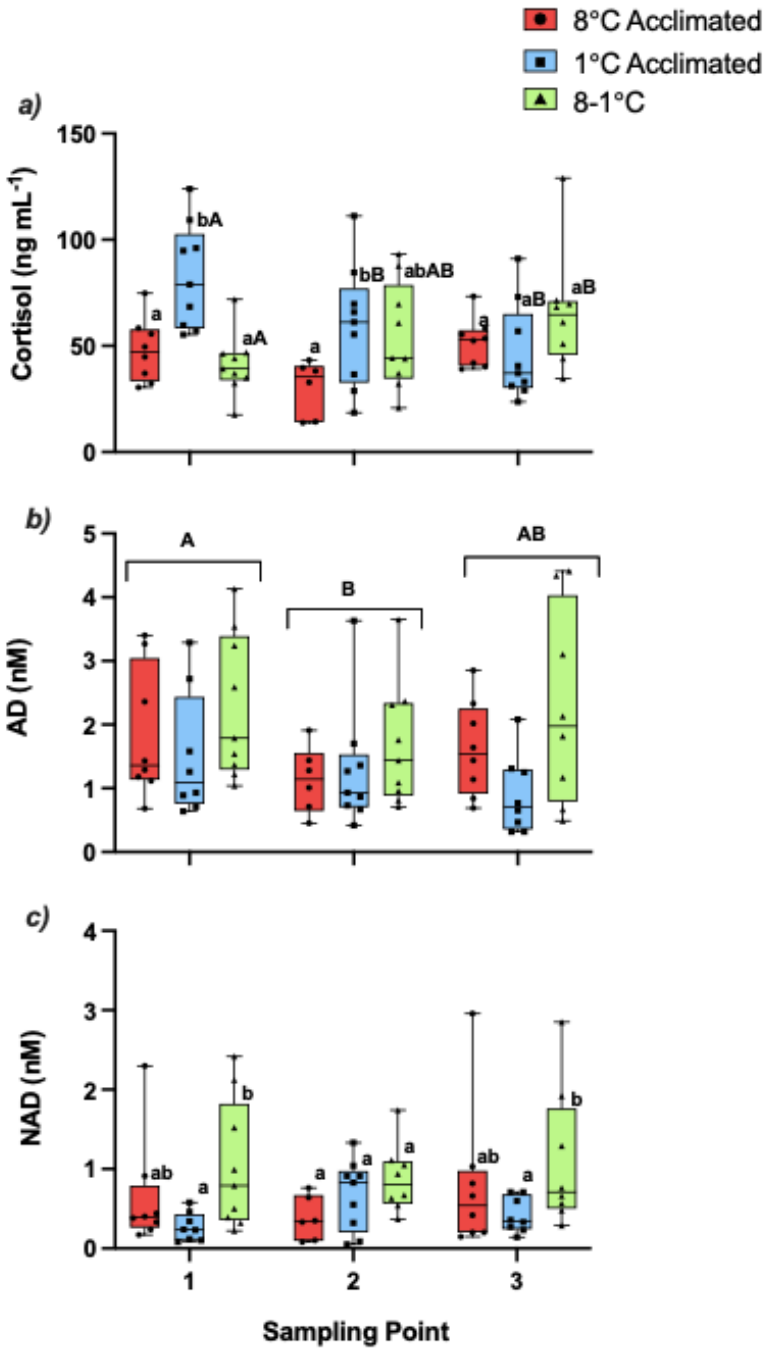

Figure 2-3. Changes in stress hormone levels in Atlantic salmon acclimated to 8 (*red*) and 1°C (*blue*), and acutely cooled from 8 to 1°C (*green*). Shown are graphs of (*a*) cortisol, (*b*) adrenaline (AD) and (*c*) noradrenaline (NAD). Symbols without a letter in common are significantly different ($p < 0.05$) between groups at a particular time point (lowercase) and between sampling points (uppercase). Values are means ± 1 s.e.m. with n = 6-9 per group.

Acclimation and sampling point also had an interactive effect ($p < 0.001$) on cortisol concentrations in these instrumented and confined fish (**Figure 2-3a**). Acclimation to 1°C resulted in significantly higher cortisol concentrations (82.6 ± 8.2 ng mL^{-1}) at the first sampling post-surgery than measured in both the 8°C acclimated and 8°C fish that were to be acutely cooled (47.9 ± 5.0 ng mL^{-1}, $p < 0.01$ and 41.2 ± 4.9 ng mL^{-1}, $p < 0.001$, respectively). Cortisol levels increased (although not significantly) 1 h after the 8 – 1°C fish reached 1°C (to 54.4 ± 8.4 ng mL^{-1}), and again to 66.2 ± 9.6 ng mL^{-1} by the third sampling point ($p < 0.05$ between the first and third samplings). Adrenaline concentrations were, on average, lower during the second sampling in all groups. However, there were no significant differences between the groups at any sampling point (**Figure 2-3b**). Noradrenaline levels were significantly higher at the first sampling (approx. 18 h post-surgery) in the fish that were to be acutely cooled as compared to the 1°C acclimated fish (**Figure 2-3c**), and this trend was consistent throughout the experiment. However, it is important to note that levels of AD and NAD concentationas were very low ([mean] < 2.5 nM) over the entire experiment.

2.3.2 Cardiorespiratory response to cold exposure

Atlantic salmon acclimated to 1°C had a significantly lower initial f_H than both the 8°C acclimated group ($p < 0.0001$) and the 8°C fish that were to be acutely cooled ($p < 0.0001$) (31.2 ± 1.4 beats min^{-1} vs 59.8 ± 2.6 beats min^{-1} and 62.3 ± 1.5 beats min^{-1}, respectively) (**Figure 2-4a**). However, there was a significant interaction between acclimation temperature and measurement time ($p < 0.0001$) on f_H (see Supplementary **Table S2**). The fish acutely exposed to decreasing temperature had a significantly lower f_H when compared to 8°C acclimated fish starting at 6°C, (52.7 ± 1.5 vs. 62.6 ± 1.4 beats min^{-1}, respectively, $p < 0.0001$), but higher values than those fish

already acclimated to 1ºC until 2ºC. At 2 and 1°C (i.e., after 6 and 7h) acutely cooled fish had f_H values of 35.7 ± 1.0 and 32.1 ± 0.8 beats min^{-1}, respectively, vs. 33.1 ± 0.5 and 32.5 ± 0.8 beats min^{-1} for 1ºC acclimated fish, and these values were not significantly different.

Stroke volume (V_S) remained relatively constant in all groups throughout the experiment (~ 0.25 mL kg^{-1}) (**Figure 2-4b**). Thus, V_S did not compensate for the decrease in f_H as the 8ºC fish were acutely cooled to 1ºC. This resulted in a significant decrease in $\dot{Q}$ in these fish after they reached 3°C (from 11.5 ± 0.8 mL min^{-1} kg^{-1} at 8°C to 9.0 ± 1.2 mL min^{-1} kg^{-1} at 3°C, $p < 0.05$: **Figure 2-4c**). This was the same temperature at which these fish had similar $\dot{Q}$ values to 1°C acclimated fish (7.2 ± 0.9 mL min^{-1} kg^{-1}, $p = 0.254$). By 1ºC, fish exposed to the gradual decrease in temperature had a $\dot{Q}$ of 7.1 ± 1.3 mL min^{-1} kg^{-1}, and this was very similar to that recorded for the 1ºC acclimated salmon at this time point (6.1 ± 0.3 mL min^{-1} kg^{-1}).

Resting values of $\dot{M}O_2$ during the first measurement were significantly lower ($p < 0.01$) in the 1°C acclimated group than both the 8°C acclimated group and the fish to be acutely cooled (27.1 ± 1.8 mg O_2 kg^{-1} hr^{-1} vs 53.6 ± 5.4 mg O_2 kg^{-1} hr^{-1} and 61.5 ± 6.3 mg O_2 kg^{-1} hr^{-1}, respectively) (**Figure 2-4d**). $\dot{M}O_2$ in the fish acutely exposed to lower temperatures decreased by 2-fold between 8 and 1ºC, and was not significantly different as compared to the 1ºC acclimated fish at this temperature (33.7 ± 5.2 vs. 24.1 ± 1.7 mg O_2 kg^{-1} hr^{-1}, respectively). $\dot{M}O_2$ / $\dot{Q}$ was not significantly affected by either the chronic or acute changes in temperature. Under all of these conditions it was approximately 0.07 ± 0.004 mg O_2 mL $blood^{-1}$ (**Figure 2-4e**).

Changes in cardiac and metabolic parameters occurred rapidly in Atlantic salmon when acutely exposed to cold temperatures (~1°C). For example, fish that were chronically acclimated to 1ºC had only slightly lower cardiorespiratory Q_{10} values for $\dot{Q}$ and f_H as compared to those acutely exposed to this temperature change (**Table 2-1**). Further, the Q_{10} values were all above

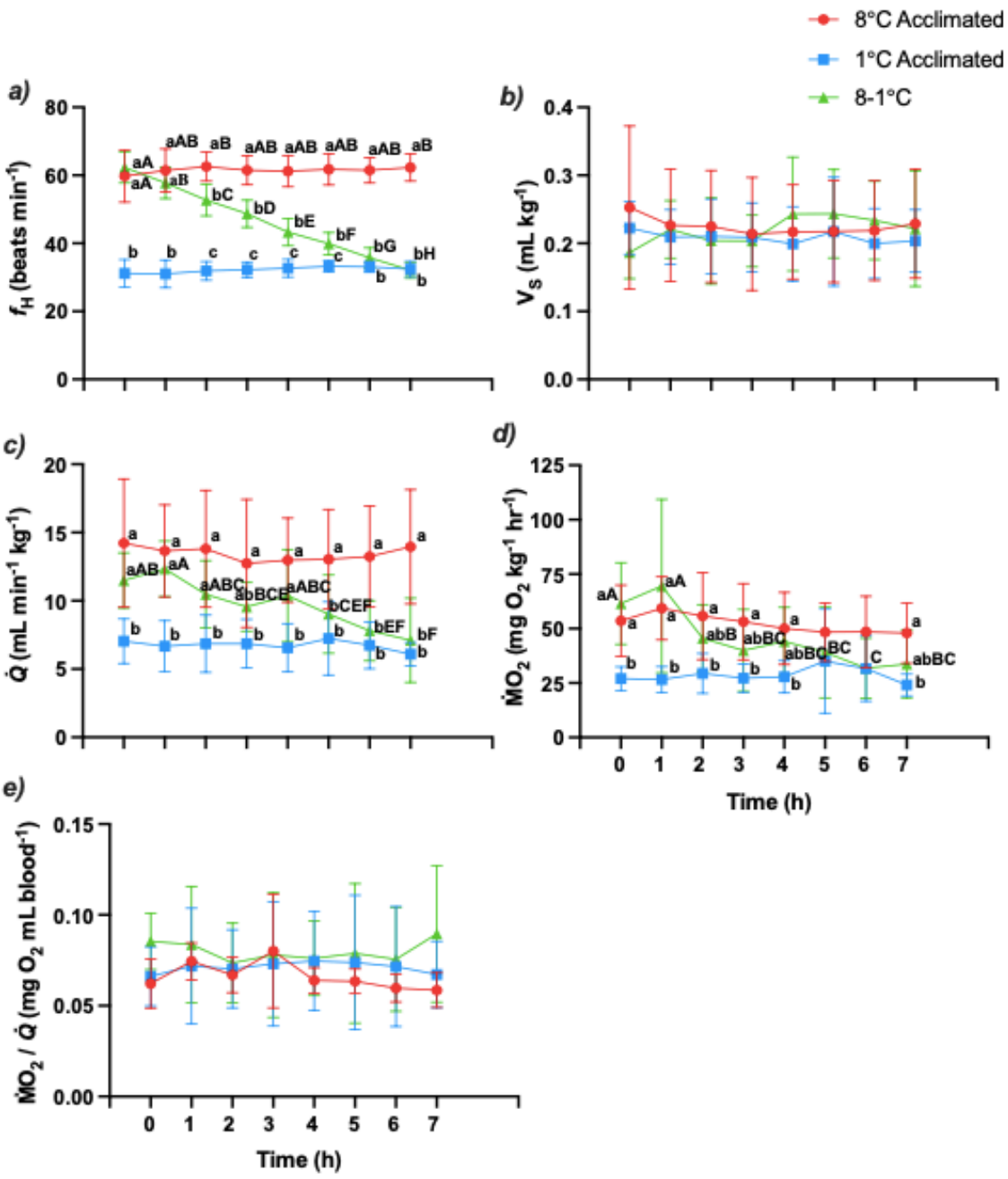

Figure 2-4. Cardiorespiratory responses of Atlantic salmon acclimated to 8 (*red*) and 1°C (*blue*), and acutely cooled from 8 to 1°C (*green*). Shown are (*a*) heart rate (f_H), (*b*) stroke volume (V_S), (*c*) cardiac output ($\dot{Q}$), (*d*) oxygen consumption ($\dot{M}O_2$), and (*e*) oxygen extraction ($\dot{M}O_2/\dot{Q}$). Symbols without a letter in common are significantly different ($p < 0.05$) between groups (lowercase) at a particular measurement time point, and between measurements time points within a group (uppercase). Values are means ± 1 s.e.m. with n = 6-9 per group.

2.5, and this suggests that there was limited thermal compensation when the salmon were acclimated to cold temperatures as compared to acutely exposed. The overnight measurements of standard metabolic rate (SMR) following the temperature change were significantly lower in both the 1 and 8 to 1°C fish as compared to the 8°C acclimated fish (**Table 2-2**). The SMR values at 8 and 1°C (~ 45 and 20 mL O_2 kg^{-1} hr^{-1}; **Table 2-2**) were approximately 67 and 85% of routine MO_2 values just prior to the injection of the pharmacological antagonists (66.5 ± 6.2; and 21.6 ± 1.5 and 28.5 ± 3.1 mL O_2 kg^{-1} hr^{-1}, respectively).

2.3.3 Neurohormonal control on the heart

Heart rate increased significantly in all groups (by ~ 4.1 beats min^{-1}; 10%) after atropine injection (the blockade of cardiac muscarinic receptors; $p < 0.01$), whereas propranolol (ß$_1$- and ß$_2$ adrenoreceptor blockade) decreased f_H by approximately 7.0 beats min^{-1} or 21% ($p < 0.0001$) (**Figure 2-5a**). The calculated cholinergic and adrenergic, 'tones' on the heart were not different between the 8°C and 1°C acclimated fish, with values ranging from ~ 12-15% to 36-53%, respectively (**Table 2-2**). However, the 8-1°C group had a significantly higher adrenergic tone (69.8 ± 9.5 %) than the 8°C acclimated fish ($p = 0.006$; **Table 2-2**). Intrinsic f_H was not significantly different between the 1°C and 8-1°C groups (23.6 ± 0.9 beats min^{-1} and 20.9 ± 1.1 beats min^{-1}, respectively), but these values were less than 50% of that measured in the 8°C acclimated group (50.5 ± 1.3 beats min^{-1}) (Q_{10} value ~ 3).

Atropine injection had no effect on $\dot{Q}$ or Vs (**Figure 2-5b and c**). Propranolol injection also had few effects on these parameters in the three groups. However, there was a significant increase in V_S after propranolol injection in the 1°C acclimated group (by 55%), and this resulted in this parameter being significantly greater in this group than in the 8-1°C group at the end of the

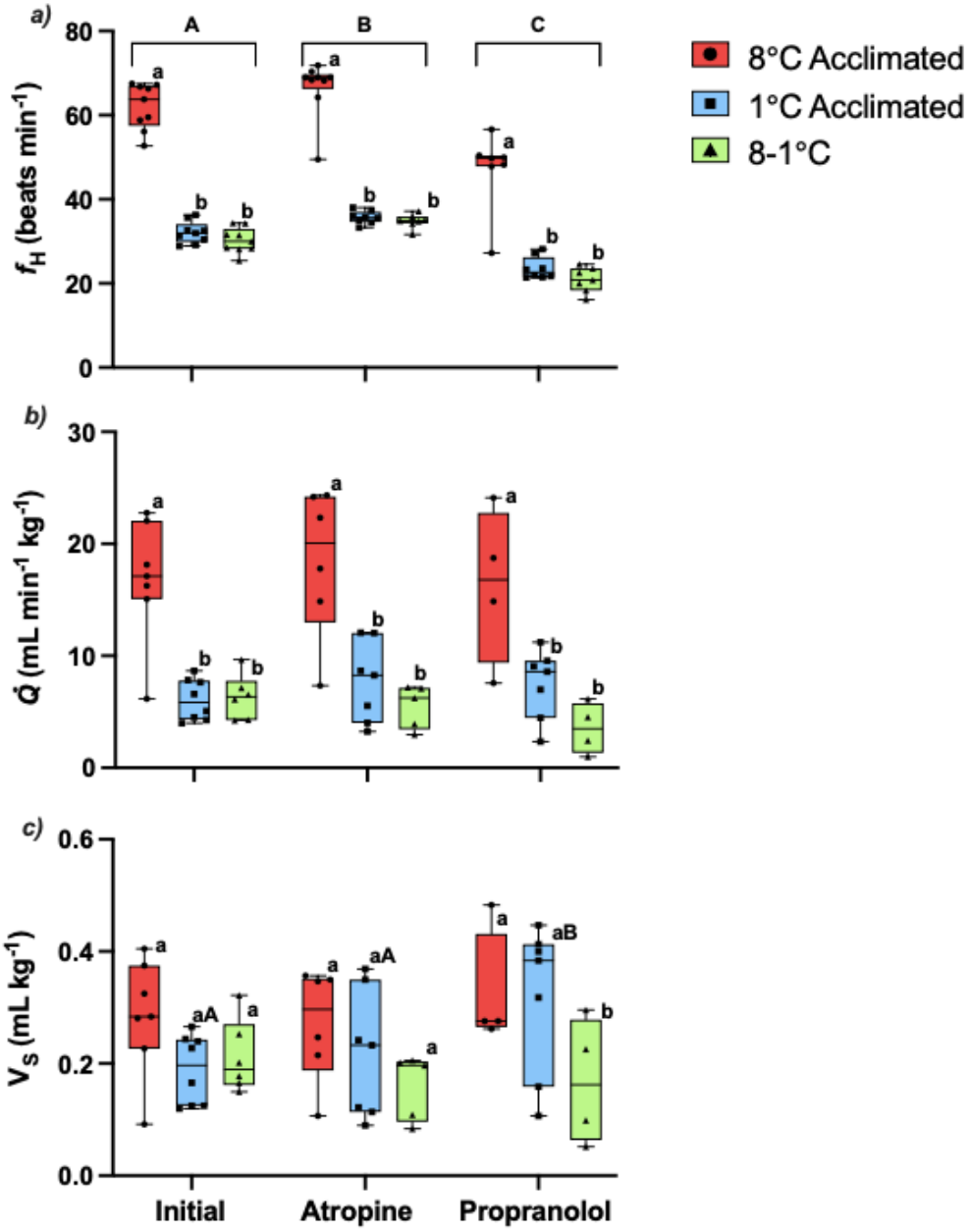

Figure 2-5. Changes in cardiac function [(*a*) heart rate (f_H), (*b*) cardiac output ($\dot{Q}$), and (c) stroke volume (V_S)] in Atlantic salmon acclimated to 8 (*red*) and 1°C (*blue*), and acutely cooled from 8 to 1°C (*green*), when injected with the pharmacological antagonists atropine sulfate (to block cholinergic nervous tone) and propranolol hydrochloride (to block ß$_1$- and ß$_2$- adrenoreceptors) Symbols without a letter in common are significantly different ($p < 0.05$) between groups (lowercase) and between the pharmacological antagonists within a group (uppercase). Values are means ± 1 s.e.m. with n = 4-9 per group.

Table 2-1. Q_{10} values for the effect of chronic cold (8 vs 1°C acclimation) and acute cold (8 to 1°C over 7h) exposure on cardiorespiratory parameters.

Q_{10}	Chronic	Acute
f_H	2.56	2.62
$\dot{Q}$	2.73	2.98
$\dot{M}O_2$	2.37	2.86
$\dot{M}O_2 / \dot{Q}$	0.90	0.94

Table 2-2. The standard and resting metabolic rate (SMR and RMR, respectively; prior to drug injections), intrinsic heart rate (f_H), and various components of 'tone' on the heart as determined using a series of pharmacological injections. Values without a letter in common are significant at $P < 0.05$. Values are means ± 1 s.e.m. with, n = 9 per group.

	8°C Acclimated	**1°C Acclimated**	**8-1°C**
Cardiorespiratory Measurements:			
SMR (mg O_2 kg^{-1} hr^{-1})	44.6 ± 5.5[a]	18.3 ± 0.7[b]	23.0 ±1.7[b]
RMR (mg O_2 kg^{-1} hr^{-1})	66.5 ± 6.2[a]	21.6 ± 1.5[b]	28.5 ± 3.1[b]
Intrinsic f_H (beats min^{-1})	50.5 ± 1.3[a]	23.6 ± 0.9[b]	20.9 ± 1.1[b]
Tone (%):			
Cholinergic	12.5 ± 4.1	15.8±4.4	17.7 ± 3.1
Adrenergic	35.7 ± 3.5[a]	52.5 ± 5.5[ab]	69.8 ± 9.5[b]

experiment (**Figure 2-5**). This result/effect was even more apparent when the raw data for V_S are shown [i.e. in volts beat^{-1}; so that fish where the calibration was not successful could be included in the analysis (**Figure S1**)]. In fact, these data show that V_S after propranolol injection was higher in the 1^{o}C acclimated group as compared to both of the other groups (**Figure S1**).

2.4 Discussion

This study adds greatly to our understanding of how the cardiorespiratory physiology of Atlantic salmon (and likely many other temperate fishes) responds when they are exposed to temperatures approaching 0^{o}C. This research shows that there are few differences in morphometric and haematological parameters, cardiac function, or the metabolism of resting Atlantic salmon (*Salmo salar*) acclimated to 1°C as compared to those acutely (over several hours) exposed to this temperature. These data suggest that there is limited thermal compensation when salmon are acclimated to, vs. acutely exposed to, temperatures approaching 0^{o}C. However, we cannot exclude the possibility that cardiorespiratory parameters 'reset' quickly following acute exposure to these temperatures (i.e. these values would be lower if exposure to 1^{o}C was much quicker; < 1 h). In addition, we report a number of important differences between the groups. First, our data show that, just like exposure to warm temperatures, V_S plays no role in temperature-dependent changes in resting cardiac function. Second, plasma cortisol levels were elevated in both groups at 1^{o}C as compared to those held at 8^{o}C, suggesting that salmon at this temperature are under increased stress. Third, although circulating catecholamine levels were not elevated in fish acutely exposed to 1^{o}C, adrenergic tone appeared to play a more prominent role in these fish with regards to the control of $f_{H,}$ and removing this tone on the heart resulted in an increase in V_S in the 1^{o}C-acclimated

fish that was not observed in the other two groups. These latter results suggest that important changes in β-adrenoreceptor sensitivity/responsiveness occur in the cardiovascular system of salmon when exposed to cold temperatures (0-1°C), but that the changes are specific to the duration of exposure.

2.4.1 Effect of cold temperatures on morphology and blood parameters

2.4.1.1 Haematocrit, haemoglobin, and RVM

An adaptation that helps to decrease blood viscosity [(a haematological parameter that increases at low/cold temperatures (Axelsson, 2005; Egginton, 1996)] is the reduction, or in extreme cases the complete absence, of Hct and Hb. These changes are well documented in polar and Antarctic fish species (Farrell and Steffensen, 2005; Holeton, 1970; Ruud, 1954). Further, to counteract the decrease in oxygen carrying capacity due to reduced Hct and [Hb], and to assist in pumping blood at low/cold temperatures, an increase in relative ventricular mass (RVM) is also a common (but not universal) observation (Aho and Vornanen, 2001; Driedzic et al., 1996; Graham and Farrell, 1989; Kent et al., 1988; Klaiman et al., 2011). For example, although rainbow trout (*Oncorhynchus mykiss*) acclimated to 5°C for 4 weeks had a 50% greater RVM than 15°C acclimated fish (Graham and Farrell, 1989), Klaiman et al. (2014) reported no differences in RVM when this species was acclimated to 4, 11 or 17°C for 8 weeks. It is very unlikely that the acclimation period of 3-8 weeks during our current experiment was not long enough to induce an increase in RVM, and it could be that the extent of the temperature decrease (to 1°C, a value much lower than the above studies) played a role. For example, a recent study (Gamperl et al., 2020) suggested that the salmonid heart's response to increased temperatures is dependent on the maximum temperature to which it is exposed; i.e., RVM only increases at warm temperatures in

salmon that are reared at temperatures very close to their upper thermal limits. Additional research, at a range of temperatures at the lower end of their thermal niche, will be required to understand how the RVM of Atlantic salmon responds to decreasing temperatures.

Blood oxygen carrying capacity (i.e., Hct and Hb) and oxygen delivery to the tissues are generally considered to be large contributors to fish thermal tolerance, particularly at warm temperatures (Anttila et al., 2013; Leeuwis et al., 2021; Muñoz et al., 2018; Pörtner and Knust, 2007; Pörtner, 2010; Wang and Overgaard, 2007). It is quite common for fish to release erythrocytes (red blood cells; RBCs) via splenic contraction within minutes of an acute stress as a secondary stress response (Muñoz et al., 2018; Pearson and Stevens, 1991), and this results in an increase in Hct (i.e., in the number and/or in size of RBCs). However, this did not occur during our experiment. This may be because SMR and RMR at 1ºC were approx. 50% of those at 8ºC (Table 2), and thus, this would have reduced the Atlantic salmon's need for blood oxygen transport.

2.4.1.2 Plasma cortisol and catecholamine levels

In the present study cortisol, a primary stress hormone released from the interrenal cells in the anterior kidney ('head kidney') due to stimulation of the hypothalamus-pituitary-interrenal (HPI) axis (Faught et al., 2016; Gamperl et al., 1994b), was elevated in fish acclimated to 1°C and acutely exposed to this temperature. These data suggest that exposure to temperatures approaching 0°C induces a stress response in Atlantic salmon, independent of the time of exposure. There is a substantial body of literature which suggests that elevated temperatures increase plasma cortisol levels in many fish species (Chadwick and McCormick, 2017; LeBlanc et al., 2011; Pérez-Casanova et al., 2008), including Atlantic salmon (Madaro et al., 2018). However, our study is the first to measure cortisol levels in Atlantic salmon acclimated and acutely exposed to extremely

low (cold) temperatures, and it suggests that temperatures approaching this species' lower thermal limit cause sublethal stress. This interpretation is consistent with data on resting Atlantic cod (*Gadus morhua*; Staurnes et al., 1980) where cortisol levels were higher after > 1 week of acclimation to 1°C as compared to ~ 8°C acclimated fish, and for common carp (*Cyprinus carpio*) exposed to cold shock (Tanck et al., 2000). Elevated levels of cortisol for prolonged periods of time are known to have detrimental physiological effects on fish via the reallocation of energy substrates, and negative tertiary effects on the immune system, growth and reproduction (Alfonso et al., 2021; Reid et al., 1998; Schreck and Tort, 2016; Wendelaar Bonga, 1997). These may even be passed on to progeny via epigenetic changes (i.e., a quaternary stress response) (Colson et al., 2019; Redfern et al., 2017). Clearly, research that addresses how temperatures near 0°C affect cortisol synthesis/production, clearance and tissue responsiveness is needed before we can understand the mechanistic basis(es) for the increased plasma [cortisol] levels, and how long-term exposure to such temperatures affects salmon stress physiology, health, and welfare.

Catecholamines (CA), predominantly adrenaline (AD) and noradrenaline (NAD) are also released into the circulation from the 'head kidney' of teleost fish as a primary response to stress (Reid et al., 1998; Schreck and Tort, 2016; Wendelaar Bonga, 1997). In this study, CA levels in all the groups and at all time points (mean plasma concentrations less than 2.5 nM) would be considered resting values (Gamperl et al., 1994b). Given the significant increase in circulating cortisol levels in this study at 1°C, and that plasma CA in rainbow trout can increase by up to ~100-fold during exposure to chronic physiological and environmental stressors (i.e., from ~ 2-5 nM to upwards of 300 nM; Gamperl et al., 1994b; LeBlanc et al., 2011, 2012; Perry et al., 1996), the absence of elevated circulating CA levels in the Atlantic salmon when acutely exposed to cold temperatures was an unexpected result. However, there is little, to no, data to which this study can

be compared. For example, although Chen et al. (2002) measured increases in plasma AD and NAD (i.e. by approx. 15 nM) in tilapia (*Oreochromis aureus*) acutely exposed to a temperature drop from 25 to 12°C (the latter close to this species' lower temperature limit), these fish were netted from their tanks prior to blood sampling, and values of AD and NAD in 'control' fish were reported to be ~ 25 and 50 nM, respectively (Chen et al., 2002).

2.4.1.3 Plasma lactate and resting metabolism

Plasma lactate is another haematological parameter that is known to increase in salmonids when exposed to thermal stress, and it is an indication that anaerobic metabolism is occurring (Clark et al., 2008; Eliason et al., 2013). For example, elevated lactate concentrations have been reported in resting Chinook (*Oncorhynchus tshawytscha*) and Sockeye (*Oncorhynchus nerka*) salmon when exposed to temperatures approaching their upper thermal limits (Clark et al., 2008; Eliason et al., 2013), and this shows that thermal stress can result in a mismatch between oxygen supply and its demand by the tissues (Pörtner and Knust, 2007; Pörtner, 2010). Interestingly, in the current experiment, acute exposure to temperatures approaching the lower limit for Atlantic salmon resulted in a decrease in plasma lactate levels. These data indicate that oxidative phosphorylation is not compromised in Atlantic salmon at temperatures close to 0°C, and is sufficient to supply the fish's metabolic needs. This finding is consistent with experiments on the effects of acclimation to 0 vs. 10°C, and an acute drop in temperature from 10 – 1°C on Atlantic salmon mitochondrial function (Gerber et al., in final prep). The Q_{10} for State 3 respiration (oxidative phosphorylation with pyruvate, malate and succinate as substrates; and with a saturating concentration of ADP) of liver mitochondria for these two groups was approximately 2.7 and 3.0. These values are very comparable to those shown in Table 1 for $\dot{M}O_2$. The mitochondria of many

fish species experience dysfunction when exposed to high temperatures due to increased proton leak due to the loss of membrane integrity, a reduced capacity for oxidative phosphorylation, a loss of ADP and substrate affinities, and increased reactive oxygen species (ROS) production (see Ekström et al., 2016b; Iftikar and Hickey, 2013; Penney et al., 2014; Gerber et al., 2020 a and b). However, our understanding of how very cold temperatures (i.e., at or close to a fish's lower thermal limit) affect mitochondrial function is extremely limited.

2.4.2 Cardiorespiratory changes upon exposure to cold temperatures

The physiological response, particularly of cardiorespiratory function, to changes in temperature vary greatly between and within species, and are influenced by the duration of exposure (e.g., acute vs. chronic)(Eliason and Anttila, 2017). Temperature coefficient (Q_{10}) values are used to describe the factorial change in a physiological rate [e.g., heart rate (f_H), cardiac output $(\dot{Q})$ and oxygen consumption ($\dot{M}O_2$)] as temperature is changed by 10°C, and are typically between 2 and 3. In the present study, the Q_{10} values for f_H, f_{Hint}, $\dot{Q}$ and $\dot{M}O_2$ were extremely similar for fish chronically exposed (acclimated) to 1°C vs. acclimated to 8°C and acutely cooled to 1°C (Table 1). Given the similar Q_{10} values between the two groups and their magnitude (2.4 – 3.0), and that acclimation normally results in fish chronically exposed to low/cold temperatures having a lower Q_{10} as compared to fish acutely exposed to these temperatures (i.e., cardiorespiratory parameters in cold-acclimated fish would be expected to be higher than in acutely exposed fish; e.g. see Aho and Vornanen, 2001; Graham, et al., 1995), one could conclude that the Atlantic salmon has a very limited capacity to acclimate to temperatures near their lower thermal limit. However, Sutcliffe (2020) reported that it takes only 8 hours for winter-acclimated trout to reset their f_H when cooled from 12 to 4ºC, and thus, it is possible that the Atlantic salmon's phenotypic

plasticity allows it to rapidly compensate for thermal effects on resting cardiorespiratory function over this temperature range; i.e., there is a rapid 'resetting' of cardiac function. This interpretation would be consistent with Lurman et al. (2012) who showed that intrinsic f_H, and the maximum performance of the *in situ* cod (*Gadus morhua*) heart, were not different between fish acclimated to 10 and 4°C, and then acutely tested at 4 and 0°C, respectively, as compared to those acclimated to these latter temperatures. Given that Q_{10} values may not be within the 'normal/typical'' range close to an animal's thermal limits, and that cardiorespiratory function has been shown to reset quite quickly in trout following thermal acclimation (Sutcliff et al., 2020), this question needs to be examined further. For example, by very quickly (i.e., < 1 h) exposing fish to the 8-1°C decrease and comparing values with 1°C acclimated fish shortly after the temperature change. If the Atlantic salmon can compensate/acclimate, or partially acclimate, to temperatures of 0-1°C, one would expect cardiorespiratory parameters after acute transfer to 0-1°C to be significantly lower than in acclimated fish.

In this study, V_S did not change with acute or chronic exposure to 1 vs. 8°C, but fish acclimated to 1°C increased V_S considerably when f_H was lowered by adrenergic blockade (Figure 5). The former result is in contrast to recent data collected on myocardial strips (Gamperl and Syme, in press) which suggests that the effects of cool temperatures on myocardial contraction would greatly constrain f_H, and that this could make increasing V_S much more favourable than increasing f_H to elevate $\dot{Q}$ at low temperatures. Given that we only assessed resting cardiorespiratory function in this study, we are planning swimming (i.e., critical swimming speed; U_{crit}) experiments designed to examine how acute and chronic exposure to 1°C affect maximum values for f_H, $\dot{Q}$, V_S and $\dot{M}O_2$. We are particularly interested in what the effects of exposure to this cold temperature have on the capacity of this species to increase f_H versus V_S.

It is difficult to reconcile why V_S increased in salmon acclimated (chronically exposed) to 1°C following β-adrenergic blockade, but not in fish that were acutely exposed to 1°C. However, the most likely explanation is an increase in filling pressure [central venous pressure (CVP), 'preload']. This is based on: the observation that the decrease in f_H following the addition of the propranolol was not vastly different between the two cold-exposed groups; and that although Sandblom and Axelsson (2007) did not find any differences in CVP in trout acclimated to 10 and 16°C (a relatively narrow temperature range, and close to the optimal temperature for this species), Sandblom and Axelsson (2005) showed that this mechanism was responsible for increasing V_S in rainbow trout during hypoxia and hypothesized that increased venous tone allows more blood to enter the central venous compartment, and therefore, increases venous pressure (Sandblom and Axelsson, 2005). Venous capacitance in fish is largely controlled by β-adrenergic mediated mechanisms (Sandblom and Axelsson, 2007). However, our results suggest that this hormonally-mediated α-adrenergic tone (which would increase tension in these vessels and decrease capacitance) was opposed by a β-adrenergic tone in 1°C-acclimated Atlantic salmon. This conclusion would be consistent with the increase in V_S following the administration of propranolol. However, propranolol has a low affinity for β_3-adrenoreceptors (Schena et al., 2019), and the blocking of β_1 and β_2-adrenoreceptors with this pharmacological agent might have enhanced β_3-adrenoreceptor-mediated lusitropic effects on the heart (i.e., improved myocardial relaxation), and thus cardiac filling. That cold (1°C) acclimation resulted in the development of a β-adrenergic tone on the venous vasculature is an interesting hypothesis that requires testing, especially given the extremely low resting levels of circulating NAD and AD measured in our fish at this temperature.

2.4.3 Important changes to cardiac control

2.4.3.1 Cholinergic vs adrenergic tone

The contribution of cholinergic and adrenergic tone to cardiac function varies widely between species and with temperature (Altimiras et al., 1997; Axelsson, 1988; Axelsson et al., 1987; Mendonca and Gamperl, 2009). For example, the cholinergic and adrenergic tones on the Atlantic cod heart at 10-12°C were 37.7% and 21.1%, respectively (Axelsson, 1988), as compared to 11.1% and 25.3% for the short-spined sculpin (*Myoxocephalus scorpius*) at 11-12°C (Axelsson et al., 1987). In the present study, cholinergic tone ~ 12-17%) was quite low in all groups. However, adrenergic tone ranged from approximately 35 – 70% between the groups, and was significantly higher in fish acutely exposed to 1°C as compared to 8°C acclimated fish (Table 2). Interestingly, the contributions of these modulators of f_H are much different than seen in Antarctic nototheniids that spend their whole life at these temperatures. The cholinergic tonus on the heart is very high under "resting" conditions (up to 80%) in this taxon, and changes in cardiac performance in this group appear to depend chiefly on modulation of this tonus (Davison et al., 1997; Egginton et al., 2006; Campbell et al., 2009). However, this may not be unexpected as the heart of these species apparently lacks adrenergic innervation, and they have low-post-stress catecholamine levels under all but the most severe stressors (Egginton et al., 2006) (i.e., the capacity to influence cardiac function via the release of catecholamines from the chromaffin cells might be limited). That the salmon acutely exposed to 1°C had an enhanced humoral adrenergic tone was somewhat surprising as circulating catecholamine levels (i.e., of both AD and NAD) were very low (~0.5 to 6 nM), and similar, in both groups exposed to 1°C. However, the fish heart is also under neural adrenergic (sympathetic) tone, and this may have been different in fish acclimated vs. acutely exposed to 1°C. This would need to be investigated by injecting fish with bretylium at least 24

hours prior to f_H measurements. Previous studies indicate that it takes $\geq$ 24 hours for this drug to be completely effective in preventing the release of catecholamines from sympathetic nerve terminals (Axelsson, 1988; Smith et al., 1985). Alternatively, exposure to 1°C for this period could have resulted in the pacemaker cells being populated by a larger number of cardiac β_2-adrenoreceptors, and/or by β_2-adrenoreceptors with lower binding affinity (i.e., increased sensitivity to AD and NAD). Changes in both cell surface β_2-adrenoceptor density and affinity have been described with cold exposure in fishes. For example, Keen et al. (1993) found that acclimation of rainbow trout to 8 vs 18°C resulted in an increase in myocardial sensitivity to adrenaline by 10-fold, and Shiels et al. (2003) reported that acute exposure of rainbow trout to cold temperatures increased the heart's adrenergic sensitivity and determined that this was a critical maintenance mechanism in fish cardiomyocytes (Shiels et al., 2003).

2.5 Conclusions and Perspectives

Overall, our results show that there are few differences in the Atlantic salmon's cardiorespiratory response when these fish are acutely exposed vs. acclimated to 1°C; a temperature they are exposed to in aquaculture cages / coastal waters in Atlantic Canada and Iceland. These data strongly suggest that these fish are able to appropriately regulate cardiac function and blood oxygen delivery when acutely exposed (over several hours) to cold temperatures, and that a loss of cardiac function/regulatory capacity is not likely related to (be the cause of) recent episodes of 'winter kill' at marine cage-sites in these areas. However, we did find that plasma cortisol levels were elevated in fish exposed to 1°C, and this suggests that fish welfare (i.e., growth, disease susceptibility etc.) could be impacted at these temperatures. Further, we did have a number of very interesting findings that should be further investigated. These include that:

(1) Resting V_S did not change when fish were acclimated or acutely exposed to 1°C, as compared to their 8°C conspecifics, and this suggests that temperature-dependent changes in $\dot{Q}$ are solely mediated by f_H across the Atlantic salmon's entire temperature range. (2) While acute exposure to 1°C results in a change in the adrenergic tone, it is unclear whether this is due to enhanced neural sympathetic tone or pacemaker β-adrenoreceptor sensitivity. (3) It appears that acclimation to this temperature alters the balance between α- and β- adrenergic control of CVP/venous capacitance, and thus, V_S. Understanding the mechanistic basis of the latter finding will require direct measurements of these parameters in fish exposed to these temperatures, as will determining whether increases in f_H and/or Vs are most important for increasing $\dot{Q}$ in fishes at these temperatures. The recent data of Gamperl and Syme (in press), based on mechanistic studies of heart function, would suggest that it is the latter. However, this hypothesis also needs experimental verification. Finally, this study was performed on male fish (the population of salmon available to us at the time), and several studies have reported that sex has a significant effect on temperature- and oxygen-dependent cardiac function and control in fishes (Clark et al., 2009; Rodnick et al., 2007; Sandblom et al., 2009). Thus, future research should include studies that include both male and female fish.

In conclusion, this research significantly improves our understanding of how salmon (fish) physiology (especially their stress and cardiorespiratory physiology) is impacted by exposure to very cold temperatures and provides important information with regards to the temperature-dependent biology of this taxon, and where research and management efforts should be focused (or not focused) with regards to improving their survival and welfare during the winter months.

2.6 References

Aho, E., Vornanen, M., 2001. Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B. 171(2), 173–179. https://doi.org/10.1007/s003600000171

Alfonso, S., Gesto, M., Sadoul, B., 2021. Temperature increase and its effects on fish stress physiology in the context of global warming. J. Fish. Biol. 98(6), 1496–1508. https://doi.org/10.1111/jfb.14599

Altimiras, J., Aissaoui, A., Tort, L., Axelsson, M., 1997. Cholinergic and adrenergic tones in the control of heart rate in teleosts. How should they be calculated? Comp. Biochem. Phys. A. 118(1), 131–139. https://doi.org/10.1016/S0300-9629(96)00402-1

Alzaid, A., Hori, T. S., Hall, J. R., Rise, M. L., Gamperl, A. K., 2015. Cold-induced changes in stress hormone and steroidogenic transcript levels in cunner (*Tautogolabrus adspersus*), a fish capable of metabolic depression. Gen. Comp. Endocr. 224, 126–135. https://doi.org/10.1016/j.ygcen.2015.07.007

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R., 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. J. Exp. Biol. 216(7), 1183–1190. https://doi.org/10.1242/jeb.080556

Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E., Farrell, A. P., 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. Nat. Commun. 5(1), 4252. https://doi.org/10.1038/ncomms5252

Axelsson, M., 1988. The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod Gadus morhua at rest and during non-exhaustive exercise. J. Exp. Biol. 137(1), 287–301.

Axelsson, M., 2005. The Circulatory System and Its Control. In: Farrell, A. P., Steffensen, J. F., (Eds.). Fish Physiology, vol 22, pp. 239–280. Elsevier. https://doi.org/10.1016/S1546-5098(04)22006-4

Axelsson, M., Ehrenström, F., Nilsson, S., 1987. Cholinergic and adrenergic influence on the teleost heart in vivo. Exp. Biol. 46(4), 179–186.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stats. Softw. 67(1), 1-48. https://doi.org/10.18637/jss.v067.i0l

Battiprolu, P. K., Harmon, K. J., Rodnick, K. J., 2007. Sex differences in energy metabolism and performance of teleost cardiac tissue. Am. J. Physiol-Reg I. 292(2), R827–R836. https://doi.org/10.1152/ajpregu.00379.2006

Beemelmanns, A., Zanuzzo, F. S., Xue, X., Sandrelli, R. M., Rise, M. L., Gamperl, A. K., 2020. The transcriptomic responses of Atlantic salmon (*Salmo salar*) to high temperature stress alone, and in combination with moderate hypoxia [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-38228/v1

Beemelmanns, A., Ribas, L., Anastasiadi, D., Moraleda-Prados, J., Zanuzzo, F. S., Rise, M. L., Gamperl, A. K., 2021. DNA methylation dynamics in Atlantic salmon (*Salmo salar*) challenged with high temperature and moderate hypoxia. Front. Mar. Sci. 7, 604878. https://doi.org/10.3389/fmars.2020.604878

Björnsson, B., Steinarsson, A., Árnason, T., 2007a. Growth model for Atlantic cod (*Gadus morhua*): Effects of temperature and body weight on growth rate. Aquaculture. 271(1–4), 216–226. https://doi.org/10.1016/j.aquaculture.2007.06.026

Björnsson, H., Olason, E. O., Jónsson, T., Henriksen, S., 2007b. Analysis of a smooth seasonal cycle with daily resolution and degree day maps for Iceland. Meteorol. Z. 16(1), 57–69. https://doi.org/10.1127/0941-2948/2007/0188

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., … Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science. 359(6371), eaam7240. https://doi.org/10.1126/science.aam7240

Brett, J. R., 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerkd*). Am. Zool. 11(1), 99–113. https://doi.org/10.1093/icb/11.1.99

Butler, P. J., Axelsson, M., Ehrenstrom, F., Metcalfe, J. D., Nilsson, S., 1989. Circulating catecholamines and swimming performance in the Atlantic cod, *Gadus Morhua*. J. Exp. Biol. 141(1), 377–387. https://doi.org/10.1242/jeb.141.1.377

Campbell, H., Davison, W., Fraser, K. P. P., Peck, L. S., Egginton, S., 2009. Heart rate and ventilation in Antarctic fishes are largely determined by ecotype. J. Fish. Biol. 74(3), 535–552. https://doi.org/10.1111/j.1095-8649.2008.02141.x

CBC News, Newfoundland, 2020, March 3. Harsh weather linked to die-off at Newfoundland salmon farm. Retrieved September 25, 2021, from: https://www.cbc.ca/news/canada/newfoundland-labrador/another-salmon-die-off-1.5483364

Chabot, D., Steffensen, J. F., Farrell, A. P., 2016. The determination of standard metabolic rate in fishes: Measuring smr in fishes. J. Fish. Biol. 88(1), 81–121. https://doi.org/10.1111/jfb.12845

Chabot, D., Zhang, Y., Farrell, A. P., 2020. Valid oxygen uptake measurements: Using high r^2 values with good intentions can bias upward the determination of standard metabolic rate. J. Fish. Biol. 98(5), 1206–1216. https://doi.org/10.1111/jfb.14650

Chadwick, J. G., McCormick, S. D., 2017. Upper thermal limits of growth in brook trout and their relationship to stress physiology. J. Exp. Biol. 220(21), 3976–3987. https://doi.org/10.1242/jeb.161224

Chen, W.-H., Sun, L.-T., Tsai, C.-L., Song, Y.-L., Chang, C.-F., 2002. Cold-stress induced the modulation of catecholamines, cortisol, immunoglobulin M, and leukocyte phagocytosis in tilapia. Gen. Comp. Endocr. 126(1), 90–100. https://doi.org/10.1006/gcen.2001.7772

Ćirić, J., 2020. 500 Tonnes of Salmon Die in Arnarlax Fish Farms. Icel. Rev. URL https://www.icelandreview.com/business/500-tonnes-of-salmon-die-in-arnarlax-fish-farms/ (accessed 2.21.21).

Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G., Farrell, A. P., 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). Am. J. Physiol-Reg. I. 295(5), 1631–1639. https://doi.org/10.1152/ajpregu.90461.2008

Clark, T. D., Hinch, S. G., Taylor, B. D., Frappell, P. B., Farrell, A. P., 2009. Sex differences in circulatory oxygen transport parameters of sockeye salmon (Oncorhynchus nerka) on the spawning ground. J. Comp. Phyiol. B. 179(5), 663–671. https://doi.org/10.1007/s00360-009-0349-1

Colson, V., Cousture, M., Damasceno, D., Valotaire, C., Nguyen, T., Le Cam, A., Bobe, J., 2019. Maternal temperature exposure impairs emotional and cognitive responses and triggers dysregulation of neurodevelopment genes in fish. PeerJ. 7, e6338. https://doi.org/10.7717/peerj.6338

Costa, I. A. S. F., Driedzic, W. R., Gamperl, A. K., 2013. Metabolic and cardiac responses of cunner *Tautogolabrus adspersus* to seasonal and acute changes in temperature. Physiol. Biochem. Zool. 86(2), 233–244. https://doi.org/10.1086/669538

Davison, W., Axelsson, M., Nilsson, S.,Forster, M. E., 1997. Cardiovascular control in Antarctic notothenioid fishes. Comp. Biol. Phys. A. 118(4), 1001–1008. https://doi.org/10.1016/S0300-9629(97)86789-8

Drost, H. E., Lo, M., Carmack, E. C., Farrell, A. P., 2016. Acclimation potential of Arctic cod (*Boreogadus saida* Lepechin) from the rapidly warming Arctic Ocean. J. Exp.Biol. 219(19). https://doi.org/10.1242/jeb.140194

Driedzic, W. R., Bailey, J. R., Sephton, D. H., 1996. Cardiac adaptations to low temperature in non-polar teleost fish. J. Exp. Biol. 275, 186–195.

Egginton, S., 1996. Blood rheology of Antarctic fishes: Viscosity adaptations at very low temperatures. J. Fish. Biol. 48(3), 513–521. https://doi.org/10.1111/j.1095-8649.1996.tb01444.x

Egginton, S., Campbell, H., Davison, W., 2006. Cardiovascular control in Antarctic fish. Deep-Sea. Res. Pt. II. 53, 1115–1130. https://doi.org/10.1016/j.dsr2.2006.03.007

Ekström, A., Hellgren, K., Gräns, A., Pichaud, N., Sandblom, E., 2016a. Dynamic changes in scope for heart rate and cardiac autonomic control during warm acclimation in rainbow trout. J. Exp. Biol. 219, 1106-1109. https://doi.org/10.1242/jeb.134312

Ekström, A., Sandblom, E., Blier, P. U., Cyr, B.-A. D., Brijs, J., Pichaud, N., 2016b. Thermal sensitivity and phenotypic plasticity of cardiac mitochondrial metabolism in European perch, *Perca fluviatilis*. J. Exp. Biol. 220, 386-296. https://doi.org/10.1242/jeb.150698

Ekström, A., Gräns, A., Sandblom, E., 2019. Can´t beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. J. Comp. Physiol. B. 189(6), 757–769. https://doi.org/10.1007/s00360-019-01243-7

Eliason, E. J., Anttila, K., 2017. Temperature and the Cardiovascular System. In: Gamperl, A. K., Gillis, T. E., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 36, pp. 235–297. Elsevier. https://doi.org/10.1016/bs.fp.2017.09.003

Eliason, E. J., Clark, T. D., Hinch, S. G., Farrell, A. P., 2013. Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. Conserv. Physiol. 1(1), cot008–cot008. https://doi.org/10.1093/conphys/cot008

Farrell, A. P., Smith, F., 2017. 4—Cardiac Form, Function and Physiology. In: Gamperl, A. K., Gillis, T. E., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 36, pp. 155–264. Academic Press. https://doi.org/10.1016/bs.fp.2017.07.001

Farrell, A. P., Steffensen, J. F., (Eds.) 2005. The Physiology of Polar Fishes. Elsevier Acad. Press, Amsterdam and Heidelberg.

Farrell, A. P., MacLeod, K. R., Chancey, B., 1986. Intrinsic mechanical properties of the perfused rainbow trout heart and the effects of catecholamines and extracellular calcium under control and acidotic conditions. J. Exp. Biol. 125(1), 319–345. https://doi.org/10.1242/jeb.125.1.319

Farrell, A. P., Eliason, E. J., Sandblom, E., Clark, T. D., 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. 87(10), 835–851. https://doi.org/10.1139/Z09-092

Farrell, A. P., Altimiras, J., Franklin, C. E., Axelsson, M., 2013. Niche expansion of the shorthorn sculpin (*Myoxocephalus scorpius*) to Arctic waters is supported by a thermal independence of cardiac performance at low temperature. Can. J. Zool. 91(8), 573–580. https://doi.org/10.1139/cjz-2013-0038

Faught, E., Aluru, N., Vijayan, M. M., 2016. The Molecular Stress Response. In: Schrek, C. B., Tort, L., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 35, pp. 113–166. Elsevier. https://doi.org/10.1016/B978-0-12-802728-8.00004-7

Fox, J., Weisberg, S., 2019. *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Franklin, C. E., Farrell, A. P., Altimiras, J., Axelsson, M., 2013. Thermal dependence of cardiac function in arctic fish: Implications of a warming world. J. Exp. Biol. 216(22), 4251–4255. https://doi.org/10.1242/jeb.087130

Frölicher, T. L., Fischer, E. M., Gruber, N., 2018. Marine heatwaves under global warming. Nature. 560(7718), 360–364. https://doi.org/10.1038/s41586-018-0383-9

Gamperl, A. K., Syme, D. A. (in Press). Can temperature-dependent changes in myocardial contractility explain why fish only increase heart rate when exposed to acute warming? J. Exp. Biol.

Gamperl, A. K., Syme, D. A., 2021. Temperature effects on the contractile performance and efficiency of oxidative muscle from a eurythermal versus a stenothermal salmonid. J. Exp. Biol. 224(15), jeb242487. https://doi.org/10.1242/jeb.242487

Gamperl, A. K., Pinder, A., Grant, R., Boutilier, R., 1994a. Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 193(1), 209–232.

Gamperl, A. K., Vijayan, M. M., Boutilier, R. G., 1994b. Experimental control of stress hormone levels in fishes: Techniques and applications. Rev. Fish. Biol. Fisher. 4(2), 215–255. https://doi.org/10.1007/BF00044129

Gamperl, A. K., Ajiboye, O. O., Zanuzzo, F. S., Sandrelli, R. M., Peroni, E. de F. C., Beemelmanns, A., 2020. The impacts of increasing temperature and moderate hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic Salmon (*Salmo salar*). Aquaculture. 519, 734874. https://doi.org/10.1016/j.aquaculture.2019.734874

Gamperl, A. K., Zrini, Z. A., Sandrelli, R. M., 2021. Atlantic Salmon (*Salmo salar*) cage-site distribution, behavior, and physiology during a Newfoundland heat wave. Front. Physiol. 12, 719594. https://doi.org/10.3389/fphys.2021.719594

Gerber, L., Clow, K. A., Gamperl, A. K., 2020a. Acclimation to warm temperatures has important implications for mitochondrial function in Atlantic salmon (*Salmo salar*). J. Exp. Biol. 224, jeb.236257. https://doi.org/10.1242/jeb.236257

Gerber, L., Clow, K. A., Mark, F. C., Gamperl, A. K., 2020b. Improved mitochondrial function in salmon (*Salmo salar*) following high temperature acclimation suggests that there are cracks in the proverbial 'ceiling.' Sci. Rep. 10(1), 21636. https://doi.org/10.1038/s41598-020-78519-4

Gilbert, M. J. H., Farrell, A. P., 2021. The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic char (Salvelinus alpinus), a northern cold-water specialist. J. Therm. Biol. 95,102816. https://doi.org/10.1016/j.jtherbio.2020.102816.

Gilbert, M. J. H., Harris, L. N., Malley, B. K., Schimnowski, A., Moore, J.-S.,Farrell, A. P., 2020. The thermal limits of cardiorespiratory performance in anadromous Arctic char (Salvelinus alpinus): A field-based investigation using a remote mobile laboratory. Conserv. Physiol. 8(1), coaa036. https://doi.org/10.1093/conphys/coaa036

Gollock, M. J., 2006. Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. J. Exp. Biol. 209(15), 2961–2970. https://doi.org/10.1242/jeb.02319

Graham, M. S., Farrell, A. P., 1989. The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. Physiol. Zool. 62(1), 38–61. https://doi.org/10.1086/physzool.62.1.30159997

Graham, M. S., Farrell, A. P., 1995. The seasonal intrinsic cardiac performance of a marine teleost. J. Exp. Biol. 118(1), 173–183.

Holeton, G. F., 1970. Oxygen uptake and circulation by a hemoglobinless antarctic fish (*Chaenocephalus aceratus Lonnberg*) compared with three red-blooded antartic fish. Comp. Biol. Physiol. 34(2), 457–471. https://doi.org/10.1016/0010-406X(70)90185-4

Huffman, J., 2019, March 17. Cooke experiences 'super chill' event off Nova Scotia coast. Retrieved September 25, 2021, from Atlantic Salmon Federation: https://www.asf.ca/news-and-magazine/salmon-news/cooke-experiences-super-chill-event-off-nova-scotia-coast

Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2017. The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. J. Exp. Biol. 220(15), 2757–2764. https://doi.org/10.1242/jeb.154021

Iftikar, F. I., Hickey, A. J. R., 2013. Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in *Notolabrus celidotus*. Plos. One. 8(5), e64120. https://doi.org/10.1371/journal.pone.0064120

IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University.

Johnson, A. C., Turko, A. J., Klaiman, J. M., Johnston, E. F., Gillis, T. E., 2014. Cold acclimation alters the connective tissue content of the zebrafish (*Danio rerio*) heart. J. Exp. Biol. 217(11), 1868–1875. https://doi.org/10.1242/jeb.101196

Johnson, N. C., Xie, S.-P., Kosaka, Y., Li, X., 2018. Increasing occurrence of cold and warm extremes during the recent global warming slowdown. Nat. Commun. 9(1), 1724. https://doi.org/10.1038/s41467-018-04040-y

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J. R., Brijs, J., Speers-Roesch, B., Roche, D. G., Gamperl, A. K., Raby, G. D., Morgan, R., Esbaugh, A. J., Gräns, A., Axelsson, M., … Clark, T. D., 2018. Oxygen- and capacity-limited thermal tolerance: Blurring ecology and physiology. J. Exp. Biol. 221(1), jeb169615. https://doi.org/10.1242/jeb.169615

Keen, A. N., Klaiman, J. M., Shiels, H. A., Gillis, T. E., 2017. Temperature-induced cardiac remodelling in fish. J. Exp. Biol. 220(2), 147–160. https://doi.org/10.1242/jeb.128496

Keen, J. E., Vianzon, D.-M., Farrell, A. P., Tibbits, G. F., 1993. Thermal acclimation alters both adrenergic sensitivity and adrenoceptor density in cardiac tissue of rainbow trout. J. Exp. Biol. 181, 21–47.

Kent, J., Koban, M., Prosser, C. L., 1988. Cold-acclimation-induced protein hypertrophy in channel catfish and green sunfish. J. Comp. Physiol. B. 158(2), 185–198. https://doi.org/10.1007/BF01075832

Killen, S. S., Christensen, E. A. F., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A., Munson, A., Nati, J. J. H., Papatheodoulou, M., McKenzie, D. J., 2021. Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. J. Exp. Biol. 224(18), jeb242522. https://doi.org/10.1242/jeb.242522

Klaiman, J. M., Fenna, A. J., Shiels, H. A., Macri, J., Gillis, T. E., 2011. Cardiac remodeling in fish: Strategies to maintain heart function during temperature change. Plos. One. 6(9), e24464. https://doi.org/10.1371/journal.pone.0024464

Klaiman, J. M., Pyle, W. G., Gillis, T. E., 2014. Cold acclimation increases cardiac myofilament function and ventricular pressure generation in trout. J. Exp. Biol. 217, 4132-4140. https://doi.org/10.1242/jeb.109041

Kuznetsova, A., Brockhoff, P. B., Christensen, R. H. B., 2017. *lmerTest Package*: Tests in Linear Mixed Effects Models. J. Stats. Softw. 82(13), 1–26. https://doi.org/10.18637/jss.v082.i13

LeBlanc, S., Middleton, S., Gilmour, K. M., Currie, S., 2011. Chronic social stress impairs thermal tolerance in the rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 214(10), 1721–1731. https://doi.org/10.1242/jeb.056135

LeBlanc, S., Höglund, E., Gilmour, K. M., Currie, S., 2012. Hormonal modulation of the heat shock response: Insights from fish with divergent cortisol stress responses. Am. J. Physiol-Reg. I. 302(1), R184–R192. https://doi.org/10.1152/ajpregu.00196.2011

Leeuwis, R. H. J., Nash, G. W., Sandrelli, R. M., Zanuzzo, F. S., Gamperl, A. K., 2019. The environmental tolerances and metabolic physiology of sablefish (*Anoplopoma fimbria*). Comp. Biochem. Phys. A. 231, 140–148. https://doi.org/10.1016/j.cbpa.2019.02.004

Leeuwis, R. H. J., Zanuzzo, F. S., Peroni, E. F. C., Gamperl, A. K., 2021. Research on sablefish (*Anoplopoma fimbria*) suggests that limited capacity to increase heart function leaves hypoxic fish susceptible to heat waves. Proc. R. Soc. B. 288(1946), 20202340. https://doi.org/10.1098/rspb.2020.2340

Liu, C., Ge, J., Zhou, Y., Thirumurugan, R., Gao, Q., Dong, S., 2020. Effects of decreasing temperature on phospholipid fatty acid composition of different tissues and hematology in Atlantic salmon (*Salmo salar*). Aquaculture. 515, 734587. https://doi.org/10.1016/j.aquaculture.2019.734587

Lurman, G. J., Petersen, L. H., Gamperl, A. K., 2012. In situ cardiac performance of Atlantic cod (*Gadus morhua*) at cold temperatures: Long-term acclimation, acute thermal challenge and the role of adrenaline. J. Exp. Biol. 215(22), 4006–4014. https://doi.org/10.1242/jeb.069849

Madaro, A., Folkedal, O., Maiolo, S., Alvanopoulou, M., Olsen, R. E., 2018. Effects of acclimation temperature on cortisol and oxygen consumption in Atlantic salmon (*Salmo salar*) post-smolt exposed to acute stress. Aquaculture. 497, 331–335. https://doi.org/10.1016/j.aquaculture.2018.07.056

Mendonca, P. C., Gamperl, A. K., 2009. Nervous and humoral control of cardiac performance in the winter flounder (*Pleuronectes americanus*). J. Exp. Biol. 212(7), 934–944. https://doi.org/10.1242/jeb.027680

Millard, S. P., 2013. *EnvStats:* An R Package for Environmental Statistics. Springer, New York. ISBN 978-1-4614-8455-4, https://www.springer.com.

Motyka, R., Norin, T., Petersen, L. H., Huggett, D. B., Gamperl, A. K., 2017. Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss)*, but does not affect their upper thermal tolerance. J. Therm. Biol. 68, 149–161. https://doi.org/10.1016/j.jtherbio.2016.03.007

Muñoz, N. J., Farrell, A. P., Heath, J. W., Neff, B. D., 2018. Hematocrit is associated with thermal tolerance and modulated by developmental temperature in juvenile chinook salmon. Physiol. Biochem. Zool. 91(1), 757–762. https://doi.org/10.1086/695556

Nikinmaa, M., Soivio, A., 1979. Oxygen dissociation curves and oxygen capacities of blood of a freshwater fish, *Salmo gairdneri*. Ann. Zool. Fenn. 16(3), 217–221. http://www.jstor.org/stable/23733642

Nilsson, S., 1983. *Autonomic Nerve Function in the Vertebrates*. Springer Berlin Heidelberg.

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., Wernberg, T., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9(1), 1324. https://doi.org/10.1038/s41467-018-03732-9

Pearson, M. P., Stevens, E. D., 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish. Physiol. Biochem. 9(1), 39–50. https://doi.org/10.1007/BF01987610

Penney, C. M., Nash, G. W., Gamperl, A. K., 2014. Cardiorespiratory responses of seawater-acclimated adult Arctic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) to an acute temperature increase. Can. J. Fish. Aquat. Sci. 71(7), 1096–1105. https://doi.org/10.1139/cjfas-2013-0569

Pérez-Casanova, J. C., Afonso, L. O. B., Johnson, S. C., Currie, S., Gamperl, A. K., 2008. The stress and metabolic responses of juvenile Atlantic cod *Gadus morhua L*. to an acute thermal challenge. J. Fish. Biol. 72(4), 899–916. https://doi.org/10.1111/j.1095-8649.2007.01763.x

Perry, S., Reid, S., Salama, A., 1996. The effects of repeated physical stress on the b-adrenergic response of the rainbow trout red blood cell. J. Exp. Biol. 199(3), 549–562. https://doi.org/10.1242/jeb.199.3.549

Pörtner, H. O., 2010. Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213(6), 881–893. https://doi.org/10.1242/jeb.037523

Pörtner, H. O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science. 315(5808), 95–97. https://doi.org/10.1126/science.1135471

R Core Team, 2021. R: A lamguage and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Redfern, J. C., Cooke, S. J., Lennox, R. J., Nannini, M. A., Wahl, D. H., Gilmour, K. M., 2017. Effects of maternal cortisol treatment on offspring size, responses to stress, and anxiety-related behavior in wild largemouth bass (*Micropterus salmoides*). Physiol. Behav. 180, 15–24. https://doi.org/10.1016/j.physbeh.2017.08.001

Reid, S. G., Bernier, N. J., Perry, S. F., 1998. The adrenergic stress response in fish: Control of catecholamine storage and release. Comp. Biochem. Phyiol. C. 120(1), 1–27. https://doi.org/10.1016/S0742-8413(98)00037-1

Rodgers, G. G., Tenzing, P., Clark, T. D., 2016. Experimental methods in aquatic respirometry: The importance of mixing devices and accounting for background respiration: methods in aquatic respirometry. J. Fish. Biol. 88(1), 65–80. https://doi.org/10.1111/jfb.12848

Ruud, J. T., 1954. Vertebrates without erythrocytes and blood pigment. Nature. 173(4410), 848–850. https://doi.org/10.1038/173848a0

Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H.-O., Duarte, C. M., Levin, L. A., Rosa, R., 2021. Impacts of hypoxic events surpass those of future ocean warming and acidification. Nat. Ecol. Evo. 5(3), 311–321. https://doi.org/10.1038/s41559-020-01370-3

Sandblom, E., Axelsson, M., 2005. Effects of hypoxia on the venous circulation in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. A. 140(2), 233–239. https://doi.org/10.1016/j.cbpb.2005.01.005

Sandblom, E., Axelsson, M., 2007. Venous hemodynamic responses to acute temperature increase in the rainbow trout (*Oncorhynchus mykiss*). Am. J. Physiol-Reg. I. 292(6), 2292-2298. https://doi.org/10.1152/ajpregu.00884.2006

Sandblom, E., Axelsson, M., 2011. Autonomic control of circulation in fish: A comparative view. Auton. Neurosci. 165(1), 127–139. https://doi.org/10.1016/j.autneu.2011.08.006

Sandblom, E., Clark, T. D., Hinch, S. G., Farrell, A. P., 2009. Sex-specific differences in cardiac control and hematology of sockeye salmon (*Oncorhynchus nerka*) approaching their spawning grounds. Am. J. Physiol-Reg I. 297(4), R1136–R1143. https://doi.org/10.1152/ajpregu.00363.2009

Sandnes, K., Lie, O., Waagbo, R., 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish. Biol. 32(1), 129–136. https://doi.org/10.1111/j.1095-8649.1988.tb05341.x

Schena, G., Caplan, M.J., 2019. Everything you always wanted to know about β_3-AR* (* but were afraid to ask). Cells. 8(4), 357. https://doi.org/10.3390/cells8040357

Schreck, C. B., Tort, L., 2016. The Concept of Stress in Fish. In: Shrek, C. B., Tort, L., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 35, pp 1–34. Elsevier. https://doi.org/10.1016/B978-0-12-802728-8.00001-1

Shiels, H. A., Vornanen, M., Farrell, A. P., 2003. Acute temperature change modulates the response of I_{Ca} to adrenergic stimulation in fish cardiomyocytes. Physiol. Biochem. Zool. 76(6), 816–824. https://doi.org/10.1086/378918

Smith, L. S., Bell, G. R., 1964. A technique for prolonged blood sampling in free-swimming salmon. J. Fish. Res. Board. Can. 21(4), 711–717. https://doi.org/10.1139/f64-064

Speers-Roesch, B., Norin, T., Driedzic, W. R., 2018. The benefit of being still: Energy savings during winter dormancy in fish come from inactivity and the cold, not from metabolic rate depression. P. Roy. Soc. B-Biol. Sci. 285(1886), 20181593. https://doi.org/10.1098/rspb.2018.1593

Staurnes, M., Rainuzzo, J. R., Sigholt, T., Jøgensen, L., 1994. Acclimation of Atlantic cod (*Gadus morhua)* to cold water: Sxtress response, osmoregulation, gill lipid composition and gill Na-K-ATPase activity. Comp. Biochem. Phys. A. 109(2), 413–421. https://doi.org/10.1016/0300-9629(94)90145-7

Sutcliffe, R. L., Li, S., Gilbert, M. J. H., Schulte, P. M., Miller, K. M., Farrell, A. P., 2020. A rapid intrinsic heart rate resetting response with thermal acclimation in rainbow trout, *Oncorhynchus mykiss*. J. Exp. Biol. 223, jeb.215210. https://doi.org/10.1242/jeb.215210

Svendsen, M. B. S., Bushnell, P. G., Steffensen, J. F., 2016. Design and setup of intermittent-flow respirometry system for aquatic organisms: How to set up an aquatic respirometry system. J. Fish. Biol. 88(1), 26–50. https://doi.org/10.1111/jfb.12797

Szekeres, P., Eliason, E., Lapointe, D., Donaldson, M., Brownscombe, J., Cooke, S., 2016. On the neglected cold side of climate change and what it means to fish. Clim. Res. 69(3), 239–245. https://doi.org/10.3354/cr01404

Tanck, M. W. T., Booms, G. H. R., Eding, E. H., Bonga, S. E. W., Komen, J., 2000. Cold shocks: A stressor for common carp. J. Fish. Biol. 57(4), 881–894. https://doi.org/10.1111/j.1095-8649.2000.tb02199.x

The Canadian Press. (2015, March 12). Extreme cold cause of fish deaths at N.S. aquaculture sites. Retrieved September 25, 2021 from CTV News: https://atlantic.ctvnews.ca/extreme-cold-cause-of-fish-deaths-at-n-s-aquaculture-sites-1.2276322

Tromp, J. J., Jones, P. L., Brown, M. S., Donald, J. A., Biro, P. A., Afonso, L. O. B., 2018. Chronic exposure to increased water temperature reveals few impacts on stress physiology and growth responses in juvenile Atlantic salmon. Aquaculture. 495, 196–204. https://doi.org/10.1016/j.aquaculture.2018.05.042

Wang, T., Overgaard, J., 2007. Ecology: The heartbreak of adapting to global warming. Science. 315(5808), 49–50. https://doi.org/10.1126/science.1137359

Wendelaar Bonga, S. E., 1997. The stress response in fish. Physiol. Rev. 77(3), 591–625. https://doi.org/10.1152/physrev.1997.77.3.591

Zanuzzo, F. S., Bailey, J. A., Garber, A. F., Gamperl, A. K., 2019. The acute and incremental thermal tolerance of Atlantic cod (*Gadus morhua*) families under normoxia and mild hypoxia. Comp. Biochem. Physiol. A. 233, 30–38. https://doi.org/10.1016/j.cbpa.2019.03.020

CHAPTER 3: Cardiorespiratory Function and Swimming Capacity of Atlantic salmon (*Salmo salar*) at Cold Temperatures

Abstract

We investigated how acclimation to 8, 4 and 1°C, and acute cooling from 8-1°C, affected the Atlantic salmon's aerobic and anaerobic metabolism, and cardiac function, during a critical swim speed (U_{crit}) test. This study revealed several interesting temperature-dependent effects. First, while differences in resting heart rate (f_H) between the groups were predictable based on previous research (range ~28 to 65 min^{-1}, with that of 8-1°C fish slightly less than 1°C-acclimated conspecifics), the former group had an ~2-fold greater resting stroke volume (V_S) as compared to the other groups, and the cardiac output ($\dot{Q}$) of 1°C-acclimated fish was much lower and compensated for by enhanced tissue oxygen extraction ($\dot{M}O_2 / \dot{Q}$). Second, increases in f_H (1.2 to 1.4-fold) contributed little to the higher $\dot{Q}$ when swum, and the contributions of $\dot{Q}$ (V_S) vs. oxygen extraction to aerobic scope (AS) were very different in the two groups tested at 1°C and reflected the available scope for these parameters. Finally, U_{crit} was 2.08 and 1.69 body lengths s^{-1} in the 8 and 4°C-acclimated groups, but only 1.27 and 1.44 in the 1°C-acclimated and 8-1°C fish, respectively; this lower value in 1°C vs. 8-1°C fish, despite higher values for maximum metabolic rate and AS, was unexpected. These data: support recent studies which suggest that the capacity to increase f_H is constrained at low temperatures; show that cardiorespiratory function at cold temperatures, and its response to increased demands, display considerable plasticity; and suggest that factors independent of oxygen delivery limit swimming capacity in salmon when chronically exposed to temperatures approaching their lower limit.

3.1 Introduction

Temperature is considered the 'master ecological factor' with regard to the biology / physiology of aquatic ectotherms (Brett, 1971), and an extensive body of work on fishes has focused on thermal relationships across various levels of biological organization [see comprehensive reviews by Farrell et al. (2009) and Eliason and Anttila (2017)]. Further, determining the optimal temperature range for key functional processes (i.e., growth, activity/swimming, reproduction etc.) has proven to be extremely useful in efforts to manage ecologically and economically important species in the current era of accelerated climate change [i.e., increases in global average temperatures, and in the frequency and severity of harmful/challenging environmental conditions/events such storms, heat waves and drastic reductions in water oxygen content (hypoxia) (Breitburg et al., 2018; Cooke et al., 2012; Horodysky et al., 2015; Killen et al., 2016; Little et al., 2020)].

It is predicted that average ocean temperatures will rise by 2 to 4°C by the end of this century (IPCC, 2018, 2022), and this has prompted in-depth research over the past several decades to understand the impacts of warm/high water temperatures on the physiology of fish (in particular salmonids) via measurements of upper thermal tolerance (Gamperl et al., 2020; Gallant et al., 2017; Leeuwis et al., 2019; Zanuzzo et al., 2019), stress physiology (i.e., behavioural changes, the release of various hormones, and changes in heat shock protein expression; Alfonso et al., 2021; Chadwick and McCormick, 2017) and cardiorespiratory (Ekström et al., 2017, 2019 and 2021; Franklin et al., 2013; Gollock et al., 2006; Leeuwis et al., 2019 and 2021; Norin et al., 2019; Steinhausen et al., 2008; Taylor et al., 1997) and neural responses (Andreassen et al., 2022). Briefly, as temperatures approach the fish's upper thermal limits, there is a greater demand for oxygen by the tissues (Clark et al., 2008; Ekström et al., 2016a; Lannig et al., 2004), and this results in increased metabolic rates and the release of various stress hormones (incl. corticosteroids

and catecholamines) to mobilize energy reserves and support cardiorespiratory function (Farrell, 1983; Faught et al., 2016). The increased oxygen demand at temperatures above a species' optimal thermal range is met in large part by increases in heart rate (f_H) which allows for greater amounts of blood to be pumped by the heart (i.e., an increase their cardiac output; $\dot{Q}$) (Eliason and Anttila, 2017; Farrell, 2009). However, disruptions in fundamental cellular and molecular functions (i.e., intrinsic/extrinsic control of cardiomyocyte and/or pacemaker cell excitability and membrane potential, and neural and mitochondrial dysfunction) limit a fish's ability to increase f_H at temperatures approaching their upper thermal limits (Andreassen et al., 2022; Ekström et al., 2021; Gerber et al., 2020; Haverinen and Vornanen, 2020; Iftikar and Hickey, 2013; Vornanen, 1998 and 2020). One of the reasons that this ability to increase f_H is such an issue for fish is that although they also possess the ability to increase the amount of blood pumped per heart beat (stroke volume; V_S) to increase blood flow/$\dot{Q}$ with increased temperatures, they do not; i.e., this has only been observed in studies where pharmacological intervention prevented increases in f_H (Gamperl et al., 2011; Keen and Gamperl, 2012).

Although research has significantly improved our understanding of fish physiology at warm temperatures, marine environments are also experiencing extreme reductions in temperatures at an increased rate, and for prolonged periods of time. These events are termed 'cold shock' or 'winter chill' (Reid et al., 2022; Szekeres et al., 2016; USGCRP, 2017), and only a few studies have investigated the cardiorespiratory responses of salmonids (temperate/eurythermal fishes) to suboptimal or extremely low/cold temperatures (0-1°C). Specifically, with the exception of the recent work by Porter et al. (2022) which described the cardiorespiratory, haematological and metabolic responses of resting Atlantic salmon to acclimation to 8 and 1°C, and when acutely exposed to a temperature change from 8-1°C, the majority of *in vivo* research on salmonid

cardiorespiratory function at cold temperatures has focused on: *i*) hypertrophy vs. hyperplasia of the myocardium/heart (i.e., an increase relative ventricular mass) (Driedzic et al., 1996; Klaiman et al., 2011); *ii*) blood flow to the red (aerobic) and white (anaerobic) muscle (Taylor et al., 1996), or *iii*) used experiments where anaesthetized fish were given pharmacological agents (atropine and isoproterenol) and exposed to very rapid acute changes in temperature (e.g., Gilbert and Farrell, 2021). Interestingly, Taylor et al (1996) also examined the effects of acclimation temperatures above and below the thermal optimum for rainbow trout on f_H (i.e., at 4, 11 and 18°C), and found that all fish had a low scope for increasing f_H with exercise. Further, Gamperl and Syme (2021) recently published data which strongly suggest that myocardial contraction/twitch kinetics greatly constrain maximal f_H at cool temperatures, and that this may underlie observations that fish elevate V_S to an equal or greater extent than f_H to meet demands for increased Q at lower temperatures.

In this study, we acclimated Atlantic salmon (*Salmo salar*) to 8, 4 and 1°C, and exposed a group of 8°C acclimated fish to an acute drop in temperature to 1°C, to examine how these long- and short-term changes in temperature near the lower limits for this species affect cardiac function, metabolic physiology, blood oxygen carrying capacity and swimming performance. The hypothesis was that an inability to increase f_H at cold temperatures would limit swimming performance, and/or result in plastic responses in other physiological mechanisms that determine blood oxygen delivery to the tissues or the fish's overall metabolic capacity (i.e., V_S, blood oxygen carrying capacity, tissue oxygen extraction ($\dot{M}O_2$ / $\dot{Q}$), anaerobic metabolism). These other mechanisms are also key determinants of fish swimming capacity at ecologically relevant temperatures (Anttila et al., 2014; Claireaux et al., 2005; Eliason et al., 2013; Keen and Farrell, 1994; Steinhausen et al., 2008), and such experiments/knowledge is crucial if we are to better predict the full range of climate change impacts on wild fish populations, as well as develop

strategies to ensure the health and welfare, and sustainable production, of fish cultured in north temperate regions.

3.2 Materials and Methods

This study was approved by the Animal Care Committee of Memorial University of Newfoundland and Labrador (protocol #21-01-KG). All procedures conducted on the salmon were performed in accordance with the Canadian Council on Animal Care's Guidelines on the 'Care and Use of Fish in Research, Teaching and Testing' (Canadian Council on Animal Care, 2005).

3.2.1 Fish husbandry and rearing conditions

Mixed sex Atlantic salmon were held in 0.8 m^3 tanks in the Annex Tank Room at the Ocean Science Center of Memorial University. The tanks were initially supplied with flow through seawater (~ 32 ppt salinity) with temperature and oxygen levels maintained at 10°C and >95% of air saturation, respectively, and with an 8 h light: 16 h dark photoperiod. There were 3 tanks containing 35 fish (~ 675 g) and after 2 weeks post-transfer, temperatures were decreased at 1°C per week to their respective acclimation temperature (8, 4 or 1°C). All fish were then held at these temperatures for ≥ 3 weeks (total acclimation ranged between 3 and 12 weeks). A custom-built glycol chiller made by Technical Services at Memorial University was used to supply the tanks with seawater < 5°C. Temperature was not lowered below 1°C as it was difficult to maintain these temperatures in the current system. All fish were fed a commercial salmon diet (5 mm, EWOS, Canada) by hand at ~1% body mass (or until satiation for fish kept at temperatures < 4°C as they exhibited reduced feeding behaviour/appetite) three times a week. Fish were fasted for a minimum of 24-48 h prior to surgery.

3.2.2 Surgical procedures and recovery

Fish were netted from their tank and anaesthetized in oxygenated seawater containing tricaine methanesulfonate (MS-222, 0.2 g L^{-1}; Syndel Laboratories Ltd., Qualicum Beach, BC, Canada) until ventilatory movements ceased. Weight (g) and fork length (cm) were measured, and then the fish were placed supine on a wetted foam pad upon a surgical table where their gills were continuously irrigated with oxygenated seawater containing a maintenance dose of MS-222 (0.1 g L^{-1}) at temperatures similar to those of acclimation (8, 4 or 1°C). Each fish was fitted with a dorsal aortic cannula (PE 50, Clay-Adams; Becton Dickensen and Co., Sparks, MD, USA) as in Smith and Bell (1964) and Gamperl et al. (1994a) to allow for blood collection at various sampling points during the experiment (see below). Salmon were then placed on their right side and a 1.3 mm diameter Doppler® flow probe (Model ES Cuff-type Transducer, 20 MHz, Iowa Doppler Products, Iowa City, IA, USA) was fitted around the ventral aorta as described in Gamperl et al. (1994a). Lastly, the flow probe lead was connected to a directional pulsed Doppler® flow meter (Model 545C-4; Bioengineering, University of Iowa, Ames, IA, USA) to ensure that the signal was of high quality, and the probe lead was secured to the the fish at 4 locations using 2-0 silk suture: to the last gill arch which is absent of gill filaments, just posterior to the pectoral fin, just below the lateral line, and just anterior to the dorsal fin.

After surgery was completed, each fish (n = 7-9 per group) was transferred to a 81 L Blazka-type swim-tunnel respirometer (University of Waterloo, Biotelemetry Institute, Waterloo, ON, USA) with an internal diameter of 25 cm and a 90 cm long working section, that was filled with water of the appropriate temperature (8, 4 or 1°C). The front of the respirometer was fitted with a plastic grid, which allowed for uniform water flow in the swimming section of the respirometer (Taylor and McPhail, 1985), and the rear of the tunnel was fitted with a stainless steel

grid that was connected to an external electrical circuit. This stainless steel grid could be electrified with a small current (~ 0.2 A; < 5 V); however, electrical stimulation to encourage swimming was only used during the experiment just prior to exhaustion as it interfered with the Doppler® flow probe signal, particularly in fish tested at 1°C. The front of the tunnel was covered with black plastic to provide the fish with a dark refuge, and to minimize stress from external stimuli (i.e., investigator presence). Seawater was supplied to the swim tunnel from a temperature-controlled 270 L water reservoir (Memorial University of Newfoundland, Technical Services), and the O_2 content of the water was maintained at > 95% air saturation by bubbling the reservoir with air.

A ~2 min "training session" was performed > 8 h post-surgery, during which the water velocity was gradually increased to ~1 body length s^{-1} (bL s^{-1}) (i.e., to induce swimming) and brought back down to 0.25 bL s^{-1} for recovery overnight. Finally, the temperature in the system was lowered to 1°C at ~1°C h^{-1} overnight for some of the 8°C acclimated fish (i.e., the acute exposure group), and maintained at this temperature until the swim trial began.

3.2.3 Critical swim speed (U_{crit}) test

Resting, active and post-exhaustion cardiorespiratory and metabolic parameters were recorded for all individual fish during a critical swim speed (U_{crit}) test (Brett, 1964). After measuring resting cardiac function and $\dot{M}O_2$ at a baseline speed of 0.25 bL s^{-1}, the swim speed was initially increased by 0.4 bL s^{-1} to induce constant swimming, followed by increments of 0.2 bL s^{-1} every 15 mins until the fish were exhausted. Exhaustion was determined as the inability of the fish to move away from/off the electric grid after 2-3 successive mild (5V) shocks and the swim speed was immediately reduced back to the baseline level (0.25 bL s^{-1}) for 1 h (**Figure 3-1**). The fish's critical swim speed was calculated as:

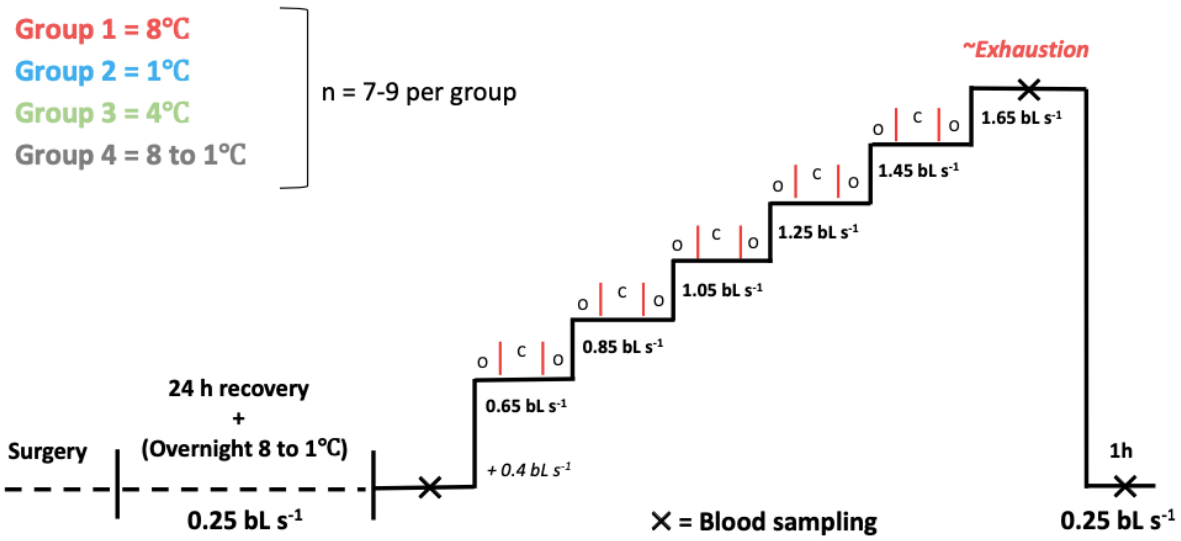

Figure 3-1. Schematic diagram of the experimental design used to determine the critical swim speed (U_{crit}) of fish exposed to cold temperatures and its effect on the salmon's cardiorespiratory and stress physiology. Three groups of fish were acclimated to 8, 4 or 1°C and tested at their respective acclimation temperature, while the last group was acclimated to 8°C and tested at 1°C (acute cold exposure). The total time at each swim speed was 15 min and was comprised of: a 5 min open (O) period; a 3-10 min closed (C) period to allow the drop in PO_2 to be recorded; and a final open (O) period to allow for % saturation to return to >85%. Cardiorespiratory parameters (f_H, V_S, $\dot{Q}$ and $\dot{M}O_2$) were recorded at rest, at each increase in swim speed, at their individual U_{crit}, and 1 h following exhaustion. Blood samples for various haematological parameters were taken as indicated by an **X** (i.e., at rest, at their U_{crit} and 1 h after exhaustion).

(1) $U_{crit} = V + [(t_f \times V_i) / t_i]$

where V = velocity at which the fish swam for the entire time increment; V_i = velocity increment (0.2 bL s^{-1}); t_f = time elapsed from the last change in current velocity to fatigue; and t_i = time increment (i.e., the time between increases in velocity or 15 min). Then, U_{crit} was corrected for the solid blocking effect of the fish (Bell and Terhune, 1970; Kline et al., 2015):

(2) $V_F = V_R (1 + \in_S)$

where V_F was the water velocity at the position of the fish's maximum girth, V_R was the water velocity at the rear the flume, and $\in_S$ was the error due to solid blocking, which was calculated as:

(3) $\in_S = \tau\lambda(A_0 / A_T)^{A_{exp}}$

where τ is a dimensionless factor for tunnel cross-sectional shape (0.8), and λ is a factor (coefficient) for the shape of the fish. The shape coefficient was set at 0.5 (i.e., the value for a fish with streamlined shape). A_0 is the cross-sectional area of the fish and was calculated as $0.25G^2\pi^{-1}$, where G was the maximum girth to the closest mm. A_T was the cross-sectional area of the swimming chamber calculated as πr^2, where the radius (r) was 100 mm, and the fractional area exponent (A_{exp}) was 1.5 (Kline et al., 2015).

3.2.4 Metabolism and cardiorespiratory function

Oxygen consumption ($\dot{M}O_2$; in mg O_2 kg^{-1} h^{-1}) was measured using intermittent closed respirometry (Sandblom et al., 2014), and using methods consistent with recommendations for aquatic respirometry as detailed in Rodgers et al. (2016), Svendsen et al. (2016) and Killen et al. (2021). Oxygen level (PO_2) in the swim tunnel was continuously measured using a fiber-optic

sensor (Dipping probe) connected to a Presens O_2 meter (PreSens Precision Sensing GmBH, Resenberg Germany). The fish's $\dot{M}O_2$ was measured by manually stopping the flow of water into the swim tunnel, and by using LabChart v8.1.5 (ADInstruments, Dunedin, New Zealand) to calculate the slope of the decrease in PO_2 after a 2 min wait period (i.e., when the tunnel was closed, which varied between 5 and 10 minutes depending on temperature to ensure an acceptable R^2). An $R^2 > 0.7$ for the decline in water O_2 level with time was used due to the large signal to noise ratio exhibited by fish at colder temperatures (i.e., at 4 and 1°C) when at rest or during measurements of RMR (see below), to ensure that $\dot{M}O_2$ was not overestimated (Chabot et al., 2020). Standard metabolic rate (SMR) was calculated by plotting the relationship between the log of metabolism [i.e., from resting metabolic rate (RMR) to maximum metabolic rate (MMR)] and swim speed (bL s^{-1}), and extrapolating back to a swim speed of 0 bL s^{-1}. Aerobic scope (AS) was then calculated as the difference between MMR and SMR. Background measurements of $\dot{M}O_2$ were made when the tunnel did not contain a fish at the end of the experiments, and these were negligible (< 1%), indicating that no substantial microbial respiration was occurring (Rodgers et al., 2016; Svendsen et al., 2016).

Heart rate (f_H) and cardiac output ($\dot{Q}$) were recorded by connecting the flow probe leads to a pulsed Doppler® flow meter and signals from the Doppler® flow meter were amplified and filtered using a data acquisition system (MP100A-CE; BIOPAC Systems, Inc., Santa Barbara, CA, USA) and a universal interface module (UIM100C, BIOPAC Systems, Inc.) connected to a laptop computer running AcqKnowledge® software (Version 3.8.2; BIOPAC Systems, Inc.). Heart rate (beats min^{-1}) was determined by measuring the number of systolic peaks during two 30s intervals while the system was "closed" and values for $\dot{Q}$ were recorded in volts (V). However, after the U_{crit} test was completed, all fish were euthanized using a lethal dose of MS-222 (0.4 g L^{-1}), and an

in situ post-mortem calibration of each flow probe was performed at physiologically relevant pressures using a peristaltic pump (MasterFlex EasyLoad®, Quebec, Canada) (Gamperl et al., 1994a) and a 'blood mimicking' solution (0.99% glycerol, 2.4% TritonX, 35% Orgasol in 200 mL of distilled water; Axelsson, pers. comm.). Briefly, following euthanization, the sinus venosus and atrium were removed, the ventricle was bisected laterally, and a steel cannula attached to the peristaltic pump tubing was tied into the ventricular lumen. This allowed the data in V to be converted to mL min^{-1} kg^{-1}, stroke volume (V_S) to be calculated as $\dot{Q} / f_H$ (in units of mL kg^{-1}) and oxygen extraction to be calculated as $\dot{M}O_2 / \dot{Q}$ (in mg O_2 mL $blood^{-1}$). In addition, the fractional change in a rate over a 10°C range (i.e., Q_{10} values) were calculated as an index of the effect of chronic (acclimation to 8 vs 1°C) and acute (8 to 1°C) temperature (T) changes on $\dot{M}O_2$, f_H, $\dot{Q}$, V_S and $\dot{M}O_2 / \dot{Q}$ (R) using the following equation:

$$(4)\ Q_{10} = \left(\frac{R2}{R1}\right)^{\left(\frac{10}{(T2-T1)}\right)}$$

Finally, after the calibration was completed, the two halves of the ventricle were weighed, and the weight, fork length, condition factor and sex were recorded for all fish used in the experiment. Finally, RVM was calculated as:

(5) RVM = [ventricular mass / fish mass) x 100]

These data are shown in **Table 3-1**, and all morphometric data were examined statistically for any significant sex effects (see **Table S4**).

Table 3-1. Morphometric data (mean ± 1 s.e.m.) for fish at each acclimation temperature. Values without a letter in common are significantly different at $p < 0.05$; n = 8-16 per group.

	8°C	**4°C**	**1°C**	**p-value**
Weight (g)	681.4 ± 19.8	721.9 ± 50.5	625.9 ± 34.5	**0.187**
Length (cm)	37.7 ± 0.4	38.0 ± 0.8	41.7 ± 0.8	**0.353**
K	1.27 ± 0.025	1.30 ± 0.020	1.23 ± 0.044	**0.334**
RVM (%)	0.0832 ± 0.00215[a]	0.0971 ± 0.00465[b]	0.0990 ± 0.00384[b]	**< 0.01***

3.2.5 Haematological parameters

Blood samples (0.5 mL) were withdrawn from the dorsal aortic cannula and immediately replaced with a 0.5 mL saline injection at three time points: 1) 24 h post-surgery (i.e., prior to an increase in swim speed); 2) Immediately following exhaustion (i.e., at their U_{crit}); and 3) 1 h after exhaustion. Blood samples were first drawn into microhaematocrit tubes and centrifuged at 10,000 x*g* for 2 min to determine haematocrit (Hct; % of red blood cells). An aliquot of 50 µL of whole blood was collected for the measurement of blood haemoglobin (Hb) concentration using the cyanomethaemoglobin method (Drabkins reagent, D5941; Sigma Aldrich, Oakville, Canada) and the absorbance was read at 540 nm using a plate reader (SpectraMax 5, Molecular Devices, San Jose, USA). Hb concentrations were calculated from standard curves using bovine Hb (Sigma, H2500). Mean cellular Hb concentration (MCHC; mg mL^{-1}) was calculated as ([Hb] / Hct) x 100. The remaining blood sample was centrifuged for 1 min at 10,000 x*g* in a mini-centrifuge (05-090-128, Fisher Scientific), and 25 µL aliquots of plasma were pipetted into Eppendorf® tubes for the measurement of plasma cortisol and lactate levels. All samples were immediately frozen in liquid N_2 and stored at -80°C. Cortisol was measured using enzyme-linked immunosorbent assay (ELISA) kits (Neogen Life Sciences, 402710, Lexington, KY, USA) and using the SpectraMax M5e microplate reader at 650 nm. Plasma lactate samples were first deproteinized with 6% (v/v) perchloric acid, then lactate was measured spectrophotometrically at 340 nm using the production of NADH/NADPH by lactate dehydrogenase (Sigma L2500), and [lactate] was calculated in reference to standard curves (Sigma L6402).

3.2.6 Statistical analyses

A Rosner's Test [*EnvStats* package in R with $\alpha = 0.05$ (Millard, 2013)] and a Grubb's Test [*outliers* package in R with $\alpha = 0.05$ (Komsta, 2022)] were used to detect outliers in all datasets prior to statistical analysis. These tests revealed that the lactate concentration from fish 7 in the 8°C acclimated group was an outlier during the third sampling point (i.e., 1 h post-exercise) and was removed from the data set. Then all data were tested for assumptions of normality and homogeneity of variance using a Shapiro-Wilks and Levene's test, respectively (Fox and Weisberg, 2019). A general linear model (lm function) and ANOVAs (anova function) were used in the *stats* package of R to assess the effect of acclimation temperature on morphometric variables [mean weight (g), fork length (cm), condition factor (K) and RVM]. Similar models were used to assess how pre-test temperature conditions affected all resting and maximum cardiorespiratory, metabolic and U_{crit} values. If there was a significant effect, a Tukey's HSD *post-hoc* test (*stats* packagein R) examined where the differences occurred. A general linear mixed model [lmer function in the *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages in R] was used to analyze all haematological parameters, including using 'fish' as a random factor, and 'group', 'sampling point' and their interaction as fixed effects. Main effects were analyzed using ANOVAs (anova function) with type III sums of squares, and if the model indicated a significant fixed effect, a Tukey's HSD *post-hoc* test identified statistical differences. All models for morphometric, cardiorespiratory, and metabolic responses were separated by sex (male vs female) and checked if there were significant effects of sex. No significant sex effects were found.

All statistical analyses were performed using Rstudio v. 1.3.1093 with R v. 4.1.0 (R Core Team, 2022). and all data in the text, and in figures and tables are means ± s.e.m. $p < 0.05$ was the

threshold used for determining statistical significance. Values displayed with an asterisk indicate $0.05 > p < 0.08$.

3.3 Results

3.3.1 Morphometric and haematological responses

There were no differences in the mean weight (676.4 ± 27.8 g), length (37.5 ± 0.4 cm) or condition factor (1.27 ± 0.021) between acclimation groups (**Table 3-1**). However, fish acclimated to both 4 and 1°C had a significantly greater relative ventricular mass (RVM) as compared to those acclimated to 8°C (0.0971 ± 0.00465 and 0.0990 ± 0.00384 vs. 0.0832 ± 0.00215 %, $p < 0.01$, respectively). No significant differences in the morphometric data were evident between male and female salmon at any acclimation temperature (**Table S5**).

There was a significant effect of both group and sampling point on haematocrit (Hct) (**Figure 3-2a**). On average, Hct (%) was lower in the 4 and 1°C acclimated salmon (by ~ 3.5%) as compared to those acclimated to 8°C and acutely cooled from 8 to 1°C. All groups had elevated Hct at their critical swim speed (U_{crit}) and 1 h post-exercise as compared to resting values, this increase ranging from 0.61 to 6.77%. There were no significant differences in haemoglobin (Hb) concentration at the various sampling points. However, on average, fish acclimated to 1°C had a significantly lower [Hb] than both the 8°C acclimated and acutely cooled fish at each sampling point (by ~ 18%; **Figure 3-2b**). No significant differences in MCHC were observed throughout this experiment, with values ranging from approx. 325 – 475 ng mL^{-1} (**Figure 3-2c**).

Plasma lactate levels increased significantly in all fish during exercise and were still elevated 1 h after the fish reached their U_{crit} ($p < 0.0001$); i.e., on average, from 0.32 ± 0.04 mM at rest to 2.14 ± 0.34 mM and 2.43 ± 0.27 mM, respectively (**Figure 3-3a**). Although there were

no significant differences in lactate levels between groups, plasma lactate levels increased by ~ 4-fold in fish acclimated to 1°C between sampling points 1 and 3, whereas the magnitude of the increase in fish acclimated to 8 and 4°C, and acutely cooled to 1°C, was ~ 14-, 7- and 9-fold, respectively. Acclimation and sampling point had an interactive effect ($p < 0.0001$) on plasma [cortisol] (**Figure 3-3b**). Resting cortisol concentrations (i.e., 24 h post-surgery and when confined within a swim tunnel) were significantly higher in fish acclimated to 1°C than both 8 and 4°C (70.6 ± 7.4 ng mL^{-1} vs 34.9 ± 6.0 ng mL^{-1} and 40.3 ± 8.7 ng mL^{-1}, $p < 0.05$, respectively). However, these values were all statistically similar to those in fish acutely cooled to 1°C (43.2 ± 11.1 ng mL^{-1}). Neither group of salmon tested at 1°C (acclimated or acutely exposed) had elevated plasma [cortisol] at exhaustion or 1 h after, with levels similar to those at rest. However, 4 and 8°C acclimated fish (which had lower resting levels) experienced 2- and 3-fold increases in plasma [cortisol] at 1 h post-exhaustion (i.e., to 100.9 ± 7.2 ng mL^{-1} and 76 ± 7.0 ng mL^{-1}, respectively).

3.3.2 Cardiac function during exercise at cold temperatures

Resting heart rate (f_H) decreased with acclimation temperature and was significantly lower in fish acutely exposed vs. acclimated to 1°C (values 64.5 ± 1.4 beats min^{-1} vs. 45.5 ± 2.2 beats min^{-1} vs. 34.1 ± 1.2 beats min^{-1} vs. 27.2 ± 1.0 beats min^{-1}, $p < 0.0001$, in 8 vs. 4 and 1 vs. 8-1°C salmon, respectively) (see **Table S6** and **Figure S3**). Maximum f_H (f_{Hmax}) during exercise was also significantly lower in colder fish (8 vs. 4 vs. 1°C); however, there was no significant difference in f_{Hmax} between the fish acclimated vs. acutely exposed to 1°C (40.6 ± 0.7 beats min^{-1} vs 37.9 ± 0.9 beats min^{-1}, respectively). Overall, fish exposed to temperatures ≤ 8°C had a very limited scope to increase f_H when challenged with the additional energic demands of exercise (average absolute

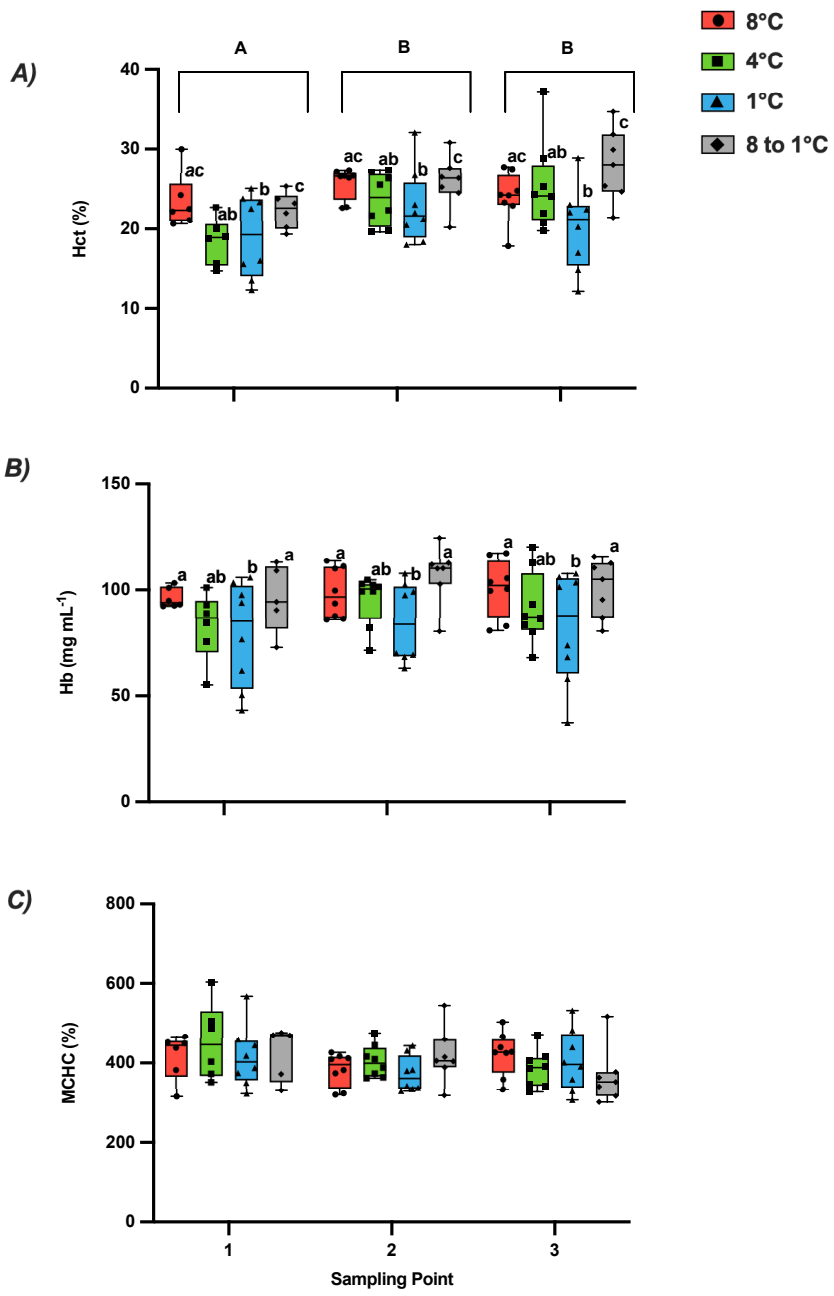

Figure 3-2. The haematological response of Atlantic salmon acclimated to 8 (*red,*●), 4 (*green,*■) and 1°C (*blue,* ▲), and acutely cooled from 8 to1°C (*grey,* ◆) prior to, at the end of, and 1 hr after a U_{crit} test. Shown are graphs for (*a*) haematocrit (Hct), (*b*) haemoglobin (Hb), and (*c*) mean cellular Hb concentration. Symbols without a letter in common are significantly different ($p < 0.05$) between groups at a particular sampling point (lowercase letters), and between sampling points within a group (uppercase letters). Values are mean ± 1 s.e.m. with n = 6-8 per group.

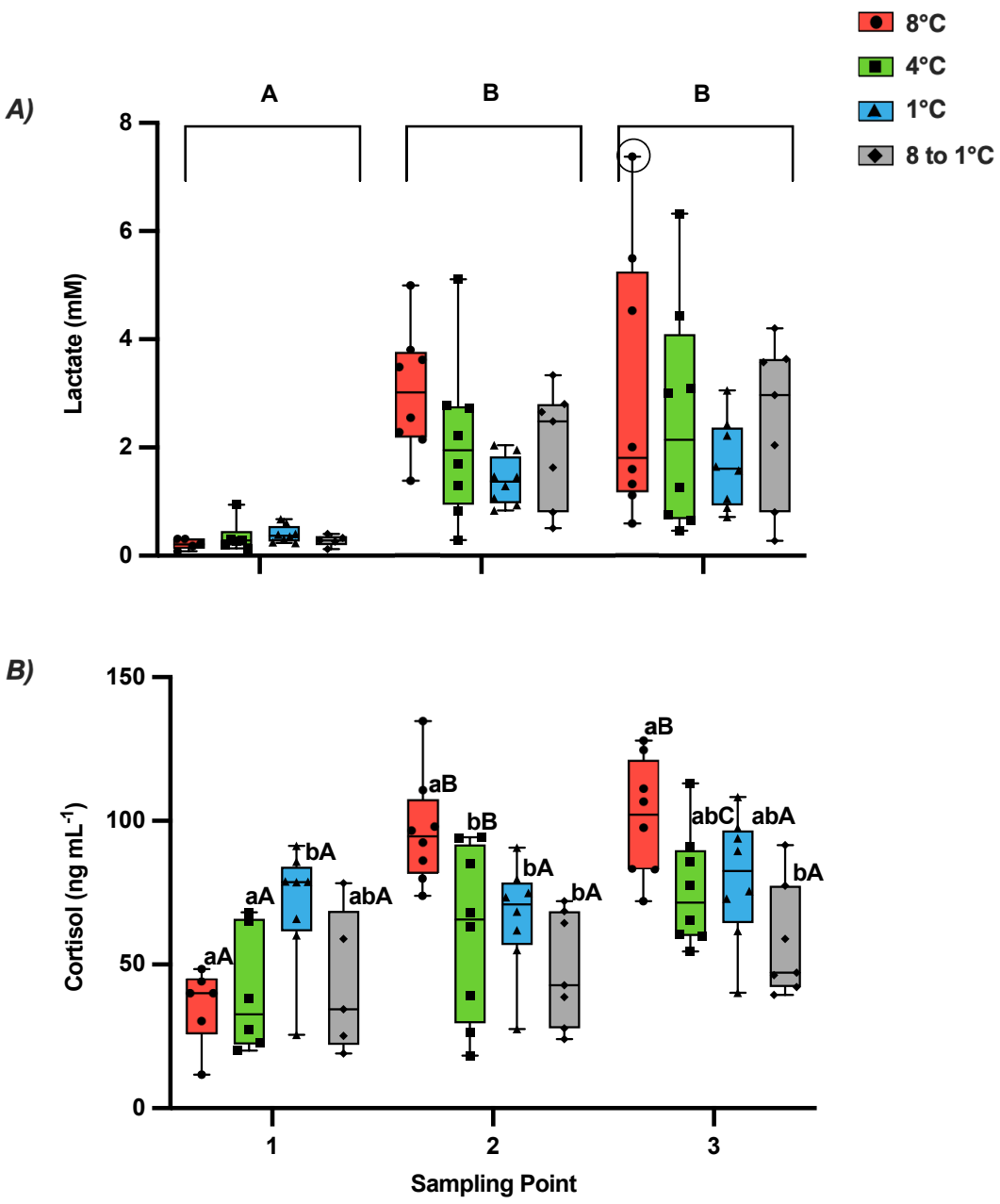

Figure 3-3. Changes in plasma lactate (*a*) and cortisol (*b*) levels in Atlantic salmon acclimated to 8 (*red,*●), 4 (*green,*■) and 1°C (*blue,*▲), and acutely cooled from 8 to1°C (*grey,*◆), prior to, and the end of, and 1 hr after a U_{crit} test. Symbols without a letter in common are significantly different ($p < 0.05$) between groups at a particular sampling point (lowercase letters) and between sampling points within a group (uppercase letters). The data point that is circled represents an outlier and was removed from statistical analyses. Values are mean ± 1 s.e.m. with n = 5-8 per group.

scope ranged from ~ 6.5 to 13.5 beats min^{-1}; **Figure 3-4a**). However, it appears that when temperature is rapidly decreased (i.e., at 1°C h^{-1}) from 8 to 1°C, salmon have a significantly larger factorial scope to increase f_H with exercise as compared to those acclimated to ≤ 8°C (**Table 3-2**). Resting cardiac output ($\dot{Q}$) was significantly lower in 1°C acclimated salmon compared to 4 and 8°C acclimated and acutely cooled fish (values 9.1 ± 0.5 vs. 17.6 ± 1.4 vs. 21.4 ± 2.1 vs. 18.7 ± 1.7 mL kg^{-1} min^{-1}, $p < 0.0001$, respectively) (**Figure 3-4b**). Further, maximum $\dot{Q}$ **(**$\dot{Q}_{max}$**)** for both 1°C acclimated and acutely exposed fish (27.8 ± 0.8 mL kg^{-1} min^{-1} and 28.7 ± 2.5 mL kg^{-1} min^{-1}, $p = 0.991$, respectively) was significantly lower, and only ~70% of the $\dot{Q}_{max}$ for 8 and 4°C acclimated fish (39.5 ± 3.1 mL kg^{-1} min^{-1} and 40.6 ± 2.7 mL kg^{-1} min^{-1}, $p = 0.936$, respectively). Interestingly, fish acclimated to 1ºC had the greatest capacity to increase $\dot{Q}$ during exercise (factorial scope of 3.11), double that measured in acutely cooled fish (1.55), and this was because: 1) these fish had a significantly higher factorial scope for V_S ($p < 0.0001$) than measured in all the other groups; and 2) resting V_S for fish acutely exposed to 1°C was comparable to the maximum V_S for 1°C acclimated fish (0.70 ± 0.08 mL kg^{-1} vs 0.70 ± 0.02 mL kg^{-1}), indicating that acutely cooled fish had a limited scope to increase their V_S during exhaustive exercise (**Figure 3-4d**).

In contrast, to the effects of acclimation to 1°C on V_S and $\dot{Q}$, chronic exposure to this temperature resulted in significantly higher values for resting oxygen extraction [$\dot{M}O_2$ / $\dot{Q}$ (0.088 ± 0.0058 mg O_2 mL $blood^{-1}$, $p < 0.0001$] in this group compared to those acclimated to 8 and 4°C (0.050 ± 0.0062 mg O_2 mL $blood^{-1}$ and 0.048 ± 0.0047 mg O_2 mL $blood^{-1}$) and to fish acutely exposed to 1°C (0.041 ± 0.0050 mg O_2 mL $blood^{-1}$). However, this group had no scope to increase oxygen extraction, and thus, the maximum value in this group was statistically similar to the other groups (mean value of 0.137 ± 0.011 mg O_2 mL $blood^{-1}$) at peak exercise; whereas fish acutely

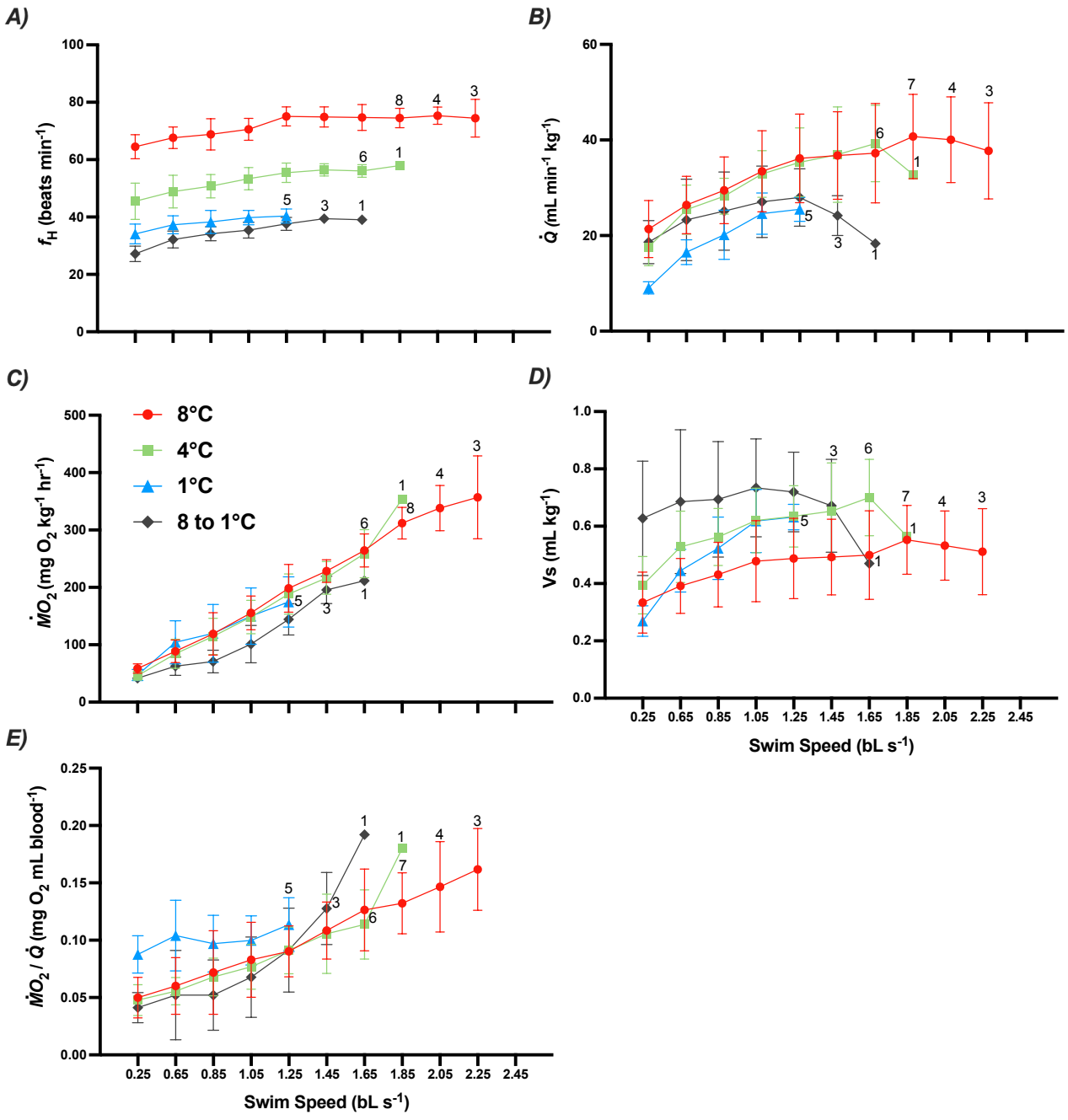

Figure 3-4. Cardiorespiratory responses of Atlantic salmon acclimated to 8 (*red,*●), 4 (*green,*■) and 1°C (*blue,* ▲), and acutely cooled from 8 to 1°C (*grey,*◆), and then given a U_{crit} test. Shown are (*a*) heart rate (f_H), (*b*) stroke volume (V_S), (*c*) oxygen consumption ($\dot{M}O_2$), (*d*) cardiac output ($\dot{Q}$) and (*e*) oxygen extraction ($\dot{M}O_2/\dot{Q}$) at each step during the critical swim speed (U_{crit}) test starting at an initial resting speed of 0.25 bL s^{-1}. Numbers above particular points indicate the remaining number of fish that made it to that swim speed within a specific group. Values are means ± 1 s.e.m. with n = 7-9 per group, unless otherwise indicated.

Table 3-2. The standard metabolic rate (SMR; mg O_2 kg^{-1} hr^{-1}), resting metabolic rate (RMR; mg O_2 kg^{-1} hr^{-1}), maximum metabolic rate (MMR; mg O_2 kg^{-1} hr^{-1}), absolute aerobic scope (AS; mg O_2 kg^{-1} hr^{-1}), critical swim speed (U_{crit}; bL s^{-1}) and factorial scope (FS) for heart rate (f_H; beats min^{-1}), cardiac output ($\dot{Q}$; mL min^{-1} kg^{-1}), stroke volume (V_S; mL kg^{-1}) and oxygen extraction ($\dot{M}O_2 / \dot{Q}$; mg O_2 mL $blood^{-1}$) of salmon acclimated to 8, 4 and 1°C, and acutely cooled from 8 to 1°C. Values without a letter in common are significantly different at $p < 0.05$ and an * indicates $0.05 > p < 0.08$. Values are means ± 1 s.e.m. with n = 7-9 per group.

	8°C	**4°C**	**1°C**	**8 to 1°C**
SMR	46.35 ± 3.29 [a]	36.12 ± 3.68 [ab]	41.70 ± 4.07 [ab]	27.57 ± 3.25 [b]
RMR	58.78 ± 2.76 [a]	46.11 ± 2.76 [b]	47.52 ± 3.51 [b*]	41.67 ± 3.05 [b]
MMR	352.08 ± 14.85 [a]	272.43 ± 16.85 [b]	222.09 ± 11.45 [c*]	184.83 ± 9.96 [c]
Absolute AS	305.72 ± 13.75 [a]	236.31 ± 16.30 [b]	185.97 ± 10.45 [c*]	147.26 ± 12.70 [c]
U_{crit}	2.08 ± 0.039 [a]	1.69 ± 0.041 [b]	1.27 ± 0.029 [c]	1.44 ± 0.037 [d]
f_H (FS)	1.21 ± 0.03 [a]	1.29 ± 0.06 [ab]	1.19 ± 0.03 [a]	1.40 ± 0.05 [b]
$\dot{Q}$ (FS)	1.98 ± 0.14 [ac]	2.42 ± 0.30 [ab]	3.11 ± 0.16 [b]	1.55 ± 0.02 [c]
V_S (FS)	1.75 ± 0.10 [a]	2.02 ± 0.31 [a]	2.68 ± 0.17 [b]	1.26 ± 0.08 [c*]
$\dot{M}O_2 / \dot{Q}$ (FS)	3.42 ± 0.26 [a]	2.86 ± 0.33 [a]	1.58 ± 0.16 [b]	2.90 ± 0.33 [a]

decreased from 8 to 1°C had a significantly greater scope (~ 2-fold) to increase $\dot{M}O_2 / \dot{Q}$ than those acclimated to 1°C when swum to exhaustion (**Table 3-2**).

Overall, these data show that acclimation and acute exposure to 1°C have very different effects on cardiorespiratory function and plasticity. The former condition results in salmon with high values of oxygen extraction at rest, and these fish meet the metabolic demands of exercise by increasing both V_S and $\dot{Q}$, whereas the opposite is true for salmon that are acutely exposed to this very low temperature. These fish have high resting values of both V_S and $\dot{Q}$, and must increase $\dot{M}O_2 / \dot{Q}$ to provide the oxygen needed to support swimming (**Figure 3-4**).

3.3.3 Differences in metabolism and swimming capacity

There were no significant differences in standard metabolic rate (SMR) between acclimation groups. However, when fish were acutely cooled from 8-1°C they had a 40 % lower SMR than fish acclimated to 8°C ($p < 0.01$), and this parameter was 35% lower than measured in 1°C acclimated fish (although this was not significant). Resting metabolic rate (RMR) was also significantly lower (by ~25%) in fish chronically and acutely exposed to cold temperatures (≤ 4°C) than when acclimated to 8°C ($p < 0.01$, **Table 3-2**). Maximum metabolic rate (MMR) was reduced significantly as acclimation/test temperature was decreased (i.e., the MMR for 4 and 1°C acclimated salmon and those acutely exposed to 1°C were only 22, 35 and 50% of those for salmon swum at 8°C). While there was no significant difference in MMR or aerobic scope between the two groups tested at 1°C ($p = 0.28$), acutely cooled fish had Q_{10} values for RMR, SMR and MMR that were consistently higher as compared to those acclimated to this temperature (**Table 3-3**).

The critical swim speed (U_{crit}) of salmon also fell as temperature decreased (i.e., in 8 vs. 4 vs. 1°C), in accordance with the data for the fish's metabolic capacity (MMR and AS). However,

Table 3-3. The Q_{10} values for the effect of chronic (8 vs. 1°C acclimation) and acute (8°C acclimation vs. 8 to 1°C acute decrease) temperature changes on resting and maximum cardiorespiratory parameters. Only mean values are shown as the 'Chronic' (acclimation) data were measured using different groups.

Q_{10}		Chronic	Acute
RMR		1.36	1.63
SMR		1.16	2.10
MMR		1.93	2.51
U_{crit}		2.02	1.69
f_H			
	Resting	2.48	3.44
	Max	2.54	2.80
$\dot{Q}$			
	Resting	3.40	1.22
	Max	1.65	1.57
V_S			
	Resting	1.36	0.37
	Max	0.70	0.57
$\dot{M}O_2$ / $\dot{Q}$			
	Resting	0.45	1.33
	Max	1.37	1.68

the swimming capacity of fish acclimated to 1°C was unexpectedly, and significantly, lower than those acutely cooled to this temperature (**Table 3-2**).

3.4 Discussion

This study examined how acclimation to temperatures approaching the Atlantic salmon's lower thermal limit (8, 4 and 1°C), and acute cold exposure (8 – 1°C), affected this species' morphometrics, cardiorespiratory function, stress response and swimming capacity. Salmon acclimated to 4 and 1°C had clearly undergone cardiac remodelling based on the higher values for relative ventricular mass in these groups as compared to 8°C fish. However, acclimation to 1°C also resulted in reduced blood haematocrit and haemoglobin levels (i.e., blood oxygen carrying capacity), and acclimation and acute exposure to this temperature resulted in elevated circulating cortisol levels [a well-established indicator of stress, (Faught et al., 2016; Gamperl et al., 1994b)]. Predictably, low temperatures (1, 4 and 8-1°C) decreased the salmon's cardiorespiratory and swimming capacity. However, this study also reports several novel findings that have important implications for our understanding of fish temperature-dependent cardiorespiratory physiology. First, increases in f_H with swimming were very limited (20 – 40%) in our salmon, and this data supports the recent findings of Gamperl et al. (2022) which suggest that myocardial contraction/twitch kinetics greatly constrain maximal f_H at cool temperatures. Second, while the higher resting f_H in 1°C-acclimated salmon as compared to those acutely exposed to this temperature was not unexpected (Aho and Vornanen, 2001; Haverinen and Vornanen, 2007; but also see below), that these two groups used completely different mechanisms to increase blood oxygen delivery / $\dot{M}O_2$ when exercised (V_S and $\dot{Q}$ vs. tissue oxygen extraction, respectively) is particularly notable. Specifically, these data show/suggest that while salmon have considerable

plasticity with regard to meeting increased oxygen demands at cold temperatures, the mechanisms evoked differ based on how long they have been in the cold (i.e., they appear to be able to increase either $\dot{Q}$ or $\dot{M}O_2$ / $\dot{Q}$ when swimming, but not both). Third, given that measures of maximum cardiac function and metabolic capacity (i.e., $\dot{Q}$, MMR and AS) were not different (or marginally higher) in salmon acclimated to 1°C as compared to fish acutely exposed to this temperature (**Table 3-2**), it is clear that factors independent of oxygen delivery limit swimming capacity in salmon when chronically exposed to temperatures approaching their lower limit. Finally, Morgan et al. (2022) recently reported that selection for faster growth in stable laboratory environments results in reduced plasticity in zebrafish (*Danio rerio*), and suggested that fish reared under such conditions are less able to counter the direct effects of temperature on key traits like metabolic rate and thermal tolerance. Indeed, a comparison of the data for resting f_H and RVM in the present study vs. Porter et al. (2022) would support such a conclusion. In contrast to this study where the fish were from commercial production stock destined for, and selected for performance in, cage-sites in the North Atlantic, Porter et al. (2022) used salmon from a commercial farm that has held salmon for 25 years (~8 generations) in land-based systems where the lowest temperature they experience is 6°C (Ignatz et al., 2022). In these latter fish, there were no differences in these important parameters between fish acclimated or acutely exposed to 1°C at rest.

3.4.1 Heart size, blood oxygen carrying capacity and stress at cold temperatures

3.4.1.1 Relative ventricular mass

In this study, salmon acclimated to 4 and 1°C had much larger (by 17-20%; **Table 3-1**) ventricles as compared to those in 8°C-acclimated fish. An increase in relative ventricular mass (RVM) has been observed in many fish populations (including non-polar temperate species) that

exploit cold water habitats during their life history. However, this is not a universal response for all fish species/populations and may differ between sexes and families (Aho and Vornanen, 2001; Axelsson, 2005; Driedzic et al., 1996; Farrell et al., 1988; Gamperl and Farrell, 2004; Graham and Farrell, 1989; Kent et al., 1988; Klaiman et al., 2011). For example, while cardiac/ventricular remodelling (i.e., hypertrophy) at cold temperatures (to support cardiac function and ensure the adequate delivery of oxygen to tissues at cold temperatures) has been well documented in many salmonids, including rainbow trout (*Oncorhynchus mykiss*), Graham and Farrell (1992) showed that both male and female rainbow trout raised in confined domesticated conditions (i.e., where temperature was held constant at 8-11°C all year) had smaller values for RVM as compared to wild fish sampled from a lake and river where temperatures ranged seasonally from 4-15°C. Further, a previous study by Porter et al. (2022), which used a population of Atlantic salmon raised in land-based systems and held at a relatively constant temperature, did not report an increase in RVM with cold acclimation, while salmon acclimated to the same temperatures in the present study (derived from a different population of salmon selected for performance in sea-cages where environmental temperatures fluctuate between 0 and 20°C) had a much greater RVM. These differing responses between salmon raised in land-based systems vs. in conditions where environmental parameters fluctuate considerably ultimately support the findings of Graham and Farrell (1992) and Morgan et al. (2022), and suggest that the phenotypic 'plasticity' of salmonids at cold temperatures may, at least in part, be related to their long-term thermal history.

3.4.1.2. Haematocrit and haemoglobin

Changes in haematology are common at cold temperatures (0-5°C), and have been well described for many polar and Antarctic species (Farrell and Steffensen, 2005; Holeton, 1970;

Ruud, 1954). An increase in blood viscosity is a common challenge for fish exposed to chronic low/cold temperatures, and this is typically counteracted by a reduction in, or in the case of some Antarctic fishes the absence of, haematocrit (Hct) and haemoglobin (Hb) (Axelsson, 2005; Egginton, 1996), which in turn lowers blood oxygen carrying capacity. However, the small decrease in haematocrit in salmon acclimated to 4 and 1°C (~3.5%) suggests that this response may not have a significant impact at cold temperatures given their low resting and maximum metabolic rates (e.g., see **Table 3-2**).

The thermal tolerance of fishes is to a large extent determined by oxygen delivery (Hct and [Hb]) to the tissues, and blood oxygen carrying capacity is particularly important in this regard when fish are exposed to acute stress or other challenges (*see Section 3.3*) (Anttila et al., 2013; Leeuwis et al., 2021; Muñoz et al., 2018; Pörtner and Knust, 2007; Pörtner, 2010; Wang and Overgaard, 2007). The release of red blood cells (RBCs) from the spleen is a common secondary stress response that enhances blood oxygen carrying capacity (Muñoz et al., 2018; Pearson and Stevens, 1991; Sandblom and Axelsson, 2007), and occurred in all groups of fish during exercise based on the elevated Hct (% RBCs); although swimming did not change [Hb] or MCHC (%). Nevertheless, it does not appear that time spent at 1°C impacts the salmon's capacity to release erythrocytes from the spleen at exhaustion (**Figure 3-2**) or to increase blood oxygen carrying capacity. The magnitude of the differences in Hct and [Hb] between fish at rest and at exhaustion were of a similar magnitude.

3.4.1.3 Elevated cortisol levels

Increased plasma concentrations of the primary stress hormone cortisol are a common indicator of sublethal stress in vertebrates due to stimulation of the hypothalamus-pituitary-

interrenal (HPI) axis (Faught et al., 2016; Gamperl et al., 1994b). Resting values for plasma cortisol (i.e., prior to the U_{crit} test) were significantly higher in salmon acclimated to 1 vs. 4 or 8°C, and this is consistent with that observed in a previous study for salmon chronically and acutely exposed to 1°C (Porter et al., 2022). Collectively, these data reinforce the notion that temperatures approaching a fish's lower thermal limit are stressful and suggests that exposure to these temperatures has consequences for the fish. The impact of a stressor is difficult to define, and typically not only indicative of the duration of stress (i.e., acute vs. chronic), but also the overall duration/severity of the consequences following exposure (Boonstra, 2013; Schreck and Tort, 2016). However, it is likely that this level of stress would affect their ability to survive additional environmental stressors (see reviews by Schreck, 2000 and Shreck and Tort, 2016) and make them more susceptible to opportunistic infections. Cortisol is immunosuppressive (Maule et al., 1987; Pickering and Pottinger, 1989; Yada and Tort, 2016) and cold temperatures alone are known to negatively impact immune function (Abram et al., 2017; Guo and Dixon, 2021). Interestingly, whereas 4 and 8^{o}C acclimated fish had elevated plasma cortisol levels at exhaustion/after during recovery, neither group of 1^{o}C exposed fish had higher plasma cortisol levels at these samplings (**Figure 3-2b**). Clearly, future research should examine the effects of cold acclimation and exposure on the secondary and tertiary stress responses of salmonids, and the possible adaptive vs. maladaptive consequences for the fish's ability to perform maximally or to return to homeostasis post-stress.

3.4.2 Cardiac function in response to cold temperatures

3.4.2.1 Acclimation effects on heart rate and its' limited scope during exercise

Changes in heart rate and the cellular components responsible for its regulation (i.e., β-adrenergic stimulation, L-type Ca^{2+} cardiomyocyte currents, sarcolemmal, Na^{+} and K^{+} currents, and ultimately the intracellular rise in free Ca^{2+} concentrations) are the most sensitive cardiac parameters to changes in temperature (Gamperl and Farrell, 2004; Graham and Farrell, 1989; Keen and Farrell, 1994; Vornanen, 1998, Vornanen et al., 2002 a and b; Vornanen, 2016; Vornanen, 2020). It has been shown that salmonids can compensate for the physiological effects of prolonged exposure (i.e., acclimation) to colder seasonal temperatures by having a higher routine heart rate (f_H) than would be observed if the fish were acutely exposed to these temperatures (Aho and Vornanen, 2001; Gamperl and Farrell, 2004; Haverinen and Vornanen, 2007). Consistent with this hypothesis, the temperature coefficient (Q_{10}) for resting f_H in the present study was lower in fish acclimated vs. acutely exposed to 1°C (**Table 3-3**), and this suggests that: 1) at least partial compensation had occurred in salmon acclimated to extremely low temperatures (i.e., approaching their lower thermal limit); and 2) the 'resetting' of heart rate *in vivo* (i.e., in a fish with intact neuroendocrine control systems) takes longer than 8 hours. This latter finding is consistent with Ekström et al. (2016b), and suggests that we must be careful interpreting data from reduced (i.e., *in vitro*) or pharmacologically manipulated preparations (e.g., Sutcliffe et al., 2020; Gilbert et al., 2022; Sandrelli and Gamperl, unpublished) with regards to how f_H changes (including its temporal nature) in free-living fish when exposed to varying environmental challenges. This study did not examine the mechanisms responsible for the resetting of heart rate, but the increase in resting f_H following acclimation to 1°C could have been the result of: a shift in cardiomyocyte membrane ion channel function and/or density (Vornanen, 1988; Vornanen et al., 2002 a and b); an increase

in adrenoreceptor sensitivity to extrinsic factors (i.e., circulating hormones adrenaline and noradrenaline) (Gamperl and Farrell, 2004); and/or in the cholinergic and adrenergic control of cardiac function (Ekström et al., 2016b; Ekström et al., 2021; Porter et al., 2022).

Interestingly, all fish regardless of acclimation or test temperature had a limited scope to increase f_H with exercise, and there was no difference in the f_{HMax} achieved between the groups tested at 1°C (**Table S6**). These results suggest that the 'resetting' of heart rate to a higher level with cold acclimation is not accompanied by an enhancement in the maximum achievable f_H. Instead, it appears that lower resting V_S (but interestingly, not the larger RVM given that V_{Smax} was not different; **Table S6**) provides salmon with a large scope for V_S which can be used to meet increased demands for cardiac pumping and oxygen delivery to the tissues. For example, 1°C acclimated fish had a scope for V_S and $\dot{Q}$ that were 2-fold that measured in those acutely exposed to this temperature, and were considerably higher than those measured in fish acclimated to/tested at 8 and 4°C (**Table 3-2**). Clearly, cold exposure regardless of duration reduced the f_{HMax} reached when salmon were exercised to exhaustion, and we now have some insights into the underlying mechanism(s) mediating this effect. For example, Gamperl et al. (2022) provide convincing data which suggests that myocardial contraction/twitch kinetics greatly constrain maximal f_H in rainbow trout at cool temperatures.

3.4.2.2 Phenotypic plasticity in salmon at temperatures approaching their lower thermal limit

The effect of cold acclimation on RVM and resting f_H, combined with the different responses of V_S and tissue oxygen extraction ($\dot{M}O_2/\dot{Q}$) during exercise between Atlantic salmon acutely vs. chronically exposed to 1°C (**Figure 3-3, Table 3-2, Table 3-3 and Table S6**), point to the considerable cardiorespiratory phenotypic plasticity that Atlantic salmon have to deal with

changes in temperature that approach their lower thermal limit. As previously discussed, all fish had a limited scope to increase f_H during exercise at ≤ 8°C. However, as acclimation temperature decreased (from 8 to 4 to 1°C), their factorial scope for V_S and $\dot{Q}$ increased (**Tables 3-2 and S6**), and thus, 1°C acclimated fish did not need to elevate $\dot{M}O_2$ / $\dot{Q}$ to meet the metabolic demands of exercise. On the contrary, fish acutely exposed to a drop in temperature to 1°C did not/could not increase V_S as this parameter was already elevated at rest, and when swum at this cold temperature increased $\dot{M}O_2$ / $\dot{Q}$ by 2.9-fold as compared to 1.6-fold in cold-acclimated fish (**Tables 2 and S6**). Surprisingly, however, one might have anticipated that the cold (1°C) acclimated salmon would have had a greater maximum V_S given their larger heart, and this value if anything was less than that observed in salmon acutely exposed to 1°C.

Interestingly, hypoxia-acclimated Atlantic salmon swimming at moderate speeds were able to increase $\dot{Q}$ and V_S to the same extent as normoxia-acclimated fish, despite their lower RVM (Harter et al., 2019), and thus, such studies raise the possibility that the increase in ventricle size when exposed to challenging environments may not be that beneficial with respect to Atlantic salmon being able to achieve maximal levels of cardiac function / pumping. However, there are other explanations with respect to why the increased RVM of 1°C acclimated salmon did not translate into a greater maximum V_S and $\dot{Q}$. It is very likely that cortisol levels were chronically elevated in the 1°C-acclimated salmon (present study, Vadboncoeur et al., 2023), and it has been reported that cortisol-induced cardiac hypertrophy is maladaptive (pathophysiological) (Johansen et al., 2017). These cortisol-induced effects could have offset any potential benefits of hypertrophic growth at low temperatures on V_S, stroke work and cardiac power output (Graham and Farrell, 1989). Alternatively, it could be that acclimation to the cold results in changes in central venous pressure (CVP) and/or its regulation, and that this limited maximum V_S in the 1°C acclimated fish.

Brijs et al. (2017) suggest that increases in CVP are critical with regard to achieving increases in end-diastolic volume, and V_S, in seawater acclimated rainbow trout. Unfortunately, there are no measurements of CVP in salmonids or other temperate fish species at temperatures below 10°C.

Acclimation to hypoxia has been reported to increase the role played by plasma-accessible carbonic anhydrase (paCA) in tissue oxygen delivery in swimming Atlantic salmon at 12°C (i.e., the role played by paCA in tissue O_2 delivery shows plasticity), and it has been suggested that maximal exercise performance in salmon may not be possible without paCA (Harter et al., 2019). However, it was not fish acclimated to 1°C that showed a large scope for $\dot{M}O_2 / \dot{Q}$; but instead it was the group that had little time to modulate paCA activity (e.g., through increases in protein levels or the isoforms expressed, especially given the cold temperatures) where large increases in $\dot{M}O_2 / \dot{Q}$ were observed. Further, it is unclear why the two groups only modulated V_S or $\dot{M}O_2 / \dot{Q}$, but not both. The latter is one of the main questions stemming from this work. Perhaps, like fish solely increasing f_H when exposed to increasing temperatures when they could achieve the same thermal tolerance and increase in $\dot{Q}$ if they instead only increased V_S (and there theoretically would be benefits to this modulation of $\dot{Q}$; Keen and Gamperl, 2012), there are inescapable constraints on physiological regulation. For example, it is possible that fish at cold temperatures cannot increase V_S and $\dot{M}O_2 / \dot{Q}$ because an increase in O_2 extraction would decrease the oxygen content of the venous blood perfusing the myocardium, and this 'feedback' prevents them from increasing together. Experiments where fish are exercised at cold temperatures while normoxic and hyperoxic would provide some insights here, as hyperoxia increases maximum $\dot{Q}$ and V_S in both temperate (McArley et al., 2021 and 2022) and tropical (Sandrelli, Porter and Gamperl, in prep) fish species.

3.4.3 Effects of cold exposure on metabolism and swimming performance

3.4.3.1 Metabolic capacity at cold temperatures

Optimal temperatures for maximum aerobic scope [i.e., the difference between standard and maximum rates of oxygen consumption ($\dot{M}O_2$)] have been well defined for fishes, with significant differences identified both between and within species when tested under various conditions (Casselman et al., 2012; Claireaux et al., 2000; Elisason and Anttila, 2017; Farrell, 2002; Hvas, et al., 2017; Pörtner, 2001; Riseth et al., 2020; Taugbøl et al., 2019). However, most of the existing literature on salmonids has focused on determining thermal optima and upper tolerance limits, and their relation to blood oxygen delivery/utilization (i.e., mitochondrial function) (Antilla et al., 2013, 2015; Beemelmanns et al., 2021; Gerber et al., 2020a and b), and this study is the first to quantify how acutely and chronically exposing this taxon to 1°C affects its' basal metabolism (i.e., SMR and RMR) and metabolic capacity (i.e., MMR and AS). The much lower values for MMR and AS in temperature-acclimated fish were anticipated as we acclimated/tested salmon at temperatures far below their optimum for performance (~13-20°C) (Brett, 1971; Handeland, et al., 2003, 2008; Hvas et al., 2017; Jonsson, et al., 2001; Keen and Farrell, 1994), and Hvas et al. (2017) showed that Atlantic salmon experience a large (~30%) reduction in aerobic scope when acclimated to 3 vs. 8°C. In our experiment, which tested salmon at even lower temperatures, we report a 40% reduction in aerobic scope when salmon are acclimated to 1°C as compared to 8°C, and this suggests that aerobic scope becomes increasingly limited at such low temperatures. That said, our data do provide evidence that acclimation to 1°C partially compensates for the effects of this temperature on the salmon's metabolism/metabolic capacity. Values for MMR and AS were 20 and 25% higher, respectively, in cold-acclimated vs. acutely exposed salmon ($0.05 > p < 0.08$). This finding is consistent with the higher State 3

respiration for liver mitochondria from 0 vs. 10ºC acclimated salmon when measured at the former temperature (Gerber et al., 2022), and with the majority of studies showing that cold-acclimation increases tissue oxidative capacity (Guderly et al., 2004; O'Brien et al., 2011).

The release of plasma lactate into the blood typically occurs at temperatures approaching the upper thermal limit of fishes as a result of a secondary stress response, or when aerobic metabolism is not sufficient to meet the fish's energetic needs (Clark et al., 2008; Eliason et al., 2013; Pörtner and Knust, 2007; Pörtner, 2010). Interestingly, studies examining the effects of cold shock on Atlantic salmon and common carp show that rapid decreases in temperature result in lower plasma lactate levels, and this suggests that fish at cold temperatures are supplied with sufficient levels of oxygen to meet their metabolic demands and that they do not experience mitochondrial dysfunction at these temperatures (Gerber et al., 2022; Porter et al., 2022; Tanck et al., 2000). In our study, resting plasma [lactate] was not different between groups, and this supports the above conclusion. A switch from aerobic to anerobic metabolism also occurs when fish reach approximately 70-80% of their U_{crit} (Burgetz et al., 1998), and as the fish swims faster there is a progressive increase in the recruitment of white (fast) muscle fibres which rely on anaerobic energy sources (Brauner et al., 2000; Farrell, 2002; Hvas et al., 2017; Rodnick and Planas, 2016; Rome et al., 1992; Steinhausen et al., 2008). In the present study, there was no significant effect of acclimation/test temperature on the increase in plasma lactate levels between fish at rest and at exhaustion, with lactate increasing from 0.32 to 2.14 mM, on average, during the U_{crit} test. This increase in lactate is very similar to that for rainbow trout when swum at 9°C (0.31 to 1.97 mM; Brauner et al., 2000). The fact that there was no difference in plasma lactate levels between the groups may seem surprising given that the U_{crit} of 1ºC fish was much lower than that of fish acclimated to 4 and 8ºC. However, when fish are exposed to cold temperatures, the white

(anaerobic) fibres are recruited at lower swimming speeds due to compromised red muscle function (Rome et al., 1985; Taylor et al., 1996), and thus, they probably make a similar overall contribution to the fish's maximum swimming capacity.

3.4.3.2 Swimming performance

Salmon in the current experiment exhibited ~20, 40 and 30% decreases in their critical swim speed (U_{crit}) when acclimated to 4 and 1°C and acutely exposed to 1°C, respectively, as compared to fish acclimated to 8°C. These reductions in U_{crit} in temperature acclimated fish are comparable to those for Atlantic salmon (30%) when acclimated to 10.5 vs 3°C (Riseth et al., 2020) and rainbow trout acclimated to 11 vs. 4°C (35%, Taylor et al., 1996), but slightly more than in Atlantic salmon acclimated to 8 vs 3°C (i.e., 12%; Hvas et al., 2017) or in wild brown trout (*Salmo trutta*) (i.e., 13%) when acclimated and tested at 5.5 vs.1.7°C (Taugbøl et al., 2019). However, it is difficult to discern why fish acclimated 1°C had a lower U_{crit} than those acutely exposed to 1°C. Their MMR and AS were similar (if not higher) despite the slight decrease in hematocrit and blood [Hb]. Acclimation to cold temperatures has been reported to result in a number of morphological and physiological changes in many species that improve the functioning of the fish's red muscle; the muscle primarily powering steady/aerobic swimming. For example, it is typically reported that as the red muscle mass and fibre size (diameter) increase, the oxidative capacity of red muscle is greater, and cold acclimation results in changes in muscle mechanics that enable the muscle to produce more power (Egginton and Cordiner, 1997; Egginton and Sidell, 1989; Guderley, 2004; Johnston et al.,1990; Jones and Sidell, 1982; Rome and Swank, 2001). Further, and importantly, Gamperl and Syme (2021) reported that the red muscle of 6°C-

acclimated salmon was able to produce more power when tested at 2ºC than that of 15ºC-acclimated fish.

Nonetheless, there are a number of potential explanations for the lower swimming performance of 1ºC-acclimated fish. Cold acclimation is concomitant with an increase in muscle mass, but the capillary density of this tissue actually decreases in rainbow trout as the increase in muscle fibre diameter is greater than the increase in the number of capillaries (Egginton and Cordiner, 1997). Further, although Barron et al. (1987) did not report changes in red muscle blood flow with cold acclimation in rainbow trout, Taylor et al. (1996) reported a dramatic (93%) decrease in blood flow to this tissue when cold vs. warm acclimated trout were swum to U_{crit}. Thus, limited perfusion of the red muscle in 1ºC-acclimated fish may have constrained its function/performance, and thus, the fish's swimming capacity. Second, this is the first study to examine the swimming performance of a salmonid acclimated to a temperature close to its lower thermal limit (1ºC), whereas the lower acclimation temperature used in previous studies was 3-4ºC (e.g., Hvas et al., 2017; Taylor et al., 1996). While this temperature difference is small (2-3ºC), Guderley and St. Pierre (1999) indicate that the there is a lack of compensation of oxidative metabolism when rainbow trout are seasonally acclimated to 0-2ºC in winter. In addition, while Crockford and Johnson (1990) showed that the force development of fast muscle fibers increased when measured at 0ºC when carp (*Cyprinus carpio* L.) were acclimated at successively cooler temperatures from 23 – 8ºC, acclimating fish to 2ºC did not improve (or actually decreased) this parameter; i.e. there was no thermal compensation when these fish were acclimated to temperatures < 8ºC. Given that the rainbow trout (another salmonid) is considered to only have a moderate acclimatory response to cold temperatures as compared to cold-adapted species (Shuman and Coughlin, 2018), it is likely that the capacity of salmonids to perform at cold temperatures

may be more limited than that of the carp. Finally, Cordiner and Egginton (1997) suggest that environmental factors other than temperature are likely to influence the nature of acclimatization in fish muscle. The carp acclimated to 2 and 5°C in Crockford and Johnson (1990) did not eat at these temperatures, and our salmon were not feeding much, if at all, at 1°C. This difference in nutritional / energetic status could have negatively influenced the swimming performance of 1°C-acclimated fish. The salmon in this experiment were all held at the same photoperiod (8 h light: 16 h dark), and it is not known how this might have affected the swimming performance of the different temperature-acclimated groups.

3.5 Conclusions and Perspectives

Overall, this study greatly extends our understanding of temperature effects on the cardiorespiratory function, metabolic capacity and swimming performance of Atlantic salmon. Our data reveal that: cold (1°C) acclimation results in an increase in RVM and resting f_H, but not f_{Hmax}; Atlantic salmon have a limited capacity to increase f_H when swimming at cold temperatures; there are key differences in how $\dot{Q}$ and $\dot{M}O_2 / \dot{Q}$ contribute to oxygen delivery to the tissues at rest and when swimming depending on whether fish are acutely (over hours) vs. chronically (> 3 weeks) exposed to temperatures approaching their lower thermal limit (0-1°C); and finally, that despite equivalent or higher values for MMR and AS, 1°C acclimated salmon had a decreased swimming performance as compared to those acutely exposed to this temperature. Further, it is apparent that, based on plasma cortisol values measured in this and other studies, salmon are chronically stressed by prolonged exposure to 1°C.

Clearly, there is a lot more research that needs to be conducted before we can understand the significance, and mechanistic underpinnings, of the above findings. To start, it would be

valuable to understand why cold-acclimated salmon did not achieve a greater maximum V_S during the U_{crit} test given that their hearts were considerably larger. Was it because the heart's function was compromised (possibly due to the pathophysiological effects of chronically elevated circulating cortisol levels), or that these fish had trouble increasing / regulating CVP and that this constrained V_S. In this regard, measurements of CVP in fish acutely and chronically exposed to cold temperatures would be particularly valuable, as such measurements have only been made on salmonids acclimated to temperatures between 10 and 16°C. It would also be important to specifically examine the effects of cold temperatures on red muscle morphological and mechanical characteristics (e.g., using cycling muscle preparations; Gamperl and Syme, 2021) given the poor swimming performance of this species when acclimated to ~ 0°C. Finally, in this study we calculate oxygen extraction indirectly as $\dot{M}O_2 / \dot{Q}$. It is key that measurements of the salmon's venous and arterial blood oxygen content be made when they are exposed to acute and chronic decreases in temperature to confirm the reported differences with regard to how $\dot{Q}$ and $\dot{M}O_2 / \dot{Q}$ were utilized to increase oxygen delivery with exercise.

3.6 References

Abram, Q., Dixon, B., Katzenbach, K., 2017. Impacts of low temperatures on the teleost immune system. Biol. 6(4), 39. https://doi.org/10.3390/biology6040039

Aho, E., Vornanen, M., 2001. Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B. 171(2), 173–179. https://doi.org/10.1007/s003600000171

Alfonso, S., Gesto, M., Sadoul, B., 2021. Temperature increase and its effects on fish stress physiology in the context of global warming. J. Fish. Biol. 98(6), 1496–1508. https://doi.org/10.1111/jfb.14599

Andreassen, A. H., Hall, P., Khatibzadeh, P., Jutfelt, F., Kermen, F., 2022. Brain dysfunction during warming is linked to oxygen limitation in larval zebrafish. Proc. Natl. Acad. Sci. 119(39), e2207052119. https://doi.org/10.1073/pnas.2207052119

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R., 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. J. Exp. Biol. 216(7), 1183–1190. https://doi.org/10.1242/jeb.080556

Antilla, K., Jørgensen, S. M., Casselman, M. T., Timmerhaus, G., Farrell, A. P., Takle, H., 2014. Association between swimming performance, cardiorespiratory morphometry, and thermal tolerance in Atlantic salmon (*Salmo salar L.*). Front. Mar. Sci. 1(76), 1-10. https://doi.org/10.3389/fmars.2014.00076

Antilla, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppänen, E., Kolari, I., Nikinmaa, M., 2015. Warm acclimation and oxygen depletion induce species-specific responses in salmonids. J. Exp. Biol. 218(10), 1471-1477. https://doi.org/10.1242/jeb.119115

Axelsson, M., 2005. The Circulatory System and Its Control. In: Farrell, A. P., Steffensen, J. F., (Eds.). Fish Physiology, vol 22, pp. 239–280. Elsevier. https://doi.org/10.1016/S1546-5098(04)22006-4

Barron, M. G., Tarr, B. D., Hayton, W. L., 1987. Temperature-dependence of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. J. Fish. Biol. 31(6), 735-744. https://doi.org/10.1111/j.1095-8649.1987.tb05276.x

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stats. Softw. 67(1), 1-48. https://doi.org/10.18637/jss.v067.i0l

Beemelmanns, A., Ribas, L., Anastasiadi, D., Moraleda-Prados, J., Zanuzzo, F. S., Rise, M. L., Gamperl, A. K., 2021. DNA methylation dynamics in Atlantic salmon (*Salmo salar*) challenged with high temperature and moderate hypoxia. Front. Mar. Sci. 7, 604878. https://doi.org/10.3389/fmars.2020.604878

Bell, W. H., Terhune, L. D B., 1970. Water tunnel design for fisheries research. J. Fish. Res. Bd. Can. Tech. Rep. 196, 1-69

Boonstra, R., 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Funct. Ecol. 27(1), 11-23. https://doi.org/10.1111/1365-2435.12008

Brauner, C. J., Thorarensen, H., Gallaugher, P., Farrell, A. P., Randall, D. J., 2000. The interaction between O_2 and CO_2 exchange in rainbow trout during graded sustained exercise. Resp. Physiol. 119(1), 83-96. https://doi.org/10.1016/S0034-5687(99)00095-X

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., … Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science. 359(6371), eaam7240. https://doi.org/10.1126/science.aam7240

Brett, J. R., 1964. The respiratory metabolism and swimming performance of young Sockeye salmon. J. Fish. Res. Bd. Can. 21(5), 1183–1226. https://doi.org/10.1139/f64-103

Brett, J. R., 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerkd*). Am. Zool. 11(1), 99-113. https://doi.org/10.1093/icb/11.1.99

Brijs, J., Sandblom, E., Dekens, E., Näslund, J., Ekström, A., Axelsson, M., 2017. Cardiac remodelling and increased central venous pressure underlie elevated stroke volume and cardiac output of seawater-acclimated rainbow trout. Am. J. Physiol-Reg. I. 312(1), R31-R39. https://doi.org/10.1152/ajpregu.00374.2016

Burgetz, I. J., Rojas-Vargas, A., Hinch, S. G., Randall, D. J., 1998. Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 201(19), 2711-2721. https://doi.org/10.1242/jeb.201.19.2711

Casselman, M. T., Anttila, K., Farrel, A. P., 2012. Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Orcorhynchus* spp. J. Fish. Biol. 80(2), 358-377. https://doi.org/10.1111/j.1095-8649.2011.03182.x

Chabot, D., Zhang, Y., Farrell, A. P., 2020. Valid oxygen uptake measurements: Using high r^2 values with good intentions can bias upward the determination of standard metabolic rate. J. Fish. Biol. 98(5), 1206–1216. https://doi.org/10.1111/jfb.14650

Chadwick, J. G., McCormick, S. D., 2017. Upper thermal limits of growth in brook trout and their relationship to stress physiology. J. Exp. Biol. 220(21), 3976–3987. https://doi.org/10.1242/jeb.161224

Claireaux, G., Webber, D. M., Lagardère, J.-P., Kerr, S. R., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). J. Sea. Res. 44(3), 257-265. https://doi.org/10.1016/S1385-1101(00)00053-8

Claireaux, G., Mackenzie, D. J., Genge, A. G., Chatelier, A., Aubin, J., Farrell, A. P., 2005. Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. J. Exp. Biol. 208(10), 1775-1784. https://doi.org/10.1242/jeb.01587

Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G., Farrell, A. P., 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). Am. J. Physiol-Reg. I. 295(5), 1631–1639. https://doi.org/10.1152/ajpregu.90461.2008

Cooke, S. J., Hinch, S. G., Donaldson, M. R., Clark, R. D., Eliason, E. J., Crossin, G. T., Raby, G. D., Jeffries, K. M., Lapointe, M., Miller, K., Patterson, D. A., Farrell, A. P., 2012. Conservation physiology in practice: how physiological knowledge has improved our ability to sustainably manage Pacific salmon during up-river migration. Phil. Trans. R. Soc. B. 367(1596), 1757-1769. https://doi.org/10.1098/rstb.2012.0022

Cordiner, S., Egginton, S., 1997. Effects of seasonal temperature acclimatization on muscle metabolism in rainbow trout, *Oncorhynchus mykiss*. J. Phys. Biochem. 16, 333-343. https://doi.org/10.1023/A:1007732003452

Crockford, T., Johnston, I.A., 1990. Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscle of the common carp (*Cyprinus carpio L.*). J. Comp. Physiol. 160(1), 23-30. https://doi.org/10.1007/BF00258759

Driedzic, W. R., Bailey, J. R., Sephton, D. H., 1996. Cardiac adaptations to low temperature in non-polar teleost fish. J. Exp. Biol. 275, 186–195. https://doi.org/10.1002/(SICI)1097-010X(19960601/15)275:2/3<186::AID-JEZ10>3.0.CO;2-I

Egginton, S., 1996. Blood rheology of Antarctic fishes: Viscosity adaptations at very low temperatures. J. Fish. Biol. 48(3), 513–521. https://doi.org/10.1111/j.1095-8649.1996.tb01444.x

Egginton, S., Cordiner, S., 1997. Cold-induced angiogenesis in seasonally acclimatized rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 200(16), 2263-2268. https://doi.org/10.1242/jeb.200.16.2263

Egginton, S., Sidell, B. D., 1989. Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. Am. J. Phys. A. 256(1), R1-R9. https://doi.org/10.1152/ajpregu.1989.256.1.R1

Ekström, A., Brijis, J., Clark, T. D., Gräns, A., Jutfelt, F., Sandblom, E., 2016a. Cardiac oxygen limitation during an acute thermal challenge in European perch: effects of chronic environmental hyperoxia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 311(2), R440-R449. https://doi.org/10.1152/ajpregu.00530.2015

Ekström, A., Hellgren, K., Gräns, A., Pichaud, N., Sandblom, E., 2016b. Dynamic changes in scope for heart rate and cardiac autonomic control during warm acclimation in rainbow trout. J. Exp. Biol. 219, 1106-1109. https://doi.org/10.1242/jeb.134312

Ekström, A., Axelsson, M., Brijs, J., Sandblom, E., 2017. Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. Am. J. Phys. Reg. I. 312(4), R549-R558. https://doi.org/10.1152/ajpregu.00536.2016

Ekström, A., Gräns, A., Sandblom, E., 2019. Can't beat the heat? Importance of cardiac control coronary perfusion for heat tolerance in rainbow trout. J. Comp. Phys. B. 189(6), 757-769. https://doi.org/10.1007/s00360-019-01243-7

Ekström, A., Sundell, E., Morgenroth, D., Sandblom, E., 2021. Andrenergic tone benefits cardiac performance and warming in two teleost fishes that lack a coronary circulation. J. Comp. Physiol. B. 191(4), 701-709. https://doi.org/10.1007/s00360-021-01359-9

Eliason, E. J., Anttila, K., 2017. Temperature and the Cardiovascular System. In: Gamperl, A. K., Gillis, T. E., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 36, pp. 235–297. Elsevier. https://doi.org/10.1016/bs.fp.2017.09.003

Eliason, E. J., Clark, T. D., Hinch, S. G., Farrell, A. P., 2013. Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. Conserv. Phys. 1(1), cot008–cot008. https://doi.org/10.1093/conphys/cot008

Farrell, A. P., 1983. A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. Can. J. Zool. 62(4), 523-536. https://doi.org/10.1139/z84-079

Farrell, A. P., 2002. Cardiorespiratory performance in salmonids during exercise at high temperatures: insight into cardiovascular design limitations in fishes. Comp. Biochem. Phys. A. 132(4), 797-810. https://doi.org/10.1016/S1095-6433(02)00049-1

Farrell, A. P., 2009. Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. J. Exp. Biol. 212, 3771-3780. https://doi.org/10.1242/jeb.023671

Farrell, A. P., Steffensen, J. F., (Eds.) 2005. The Physiology of Polar Fishes. Elsevier Academic Press, Amsterdam and Heidelberg

Farrell, A. P., Hammons, A. M., Graham, M. S., Tibbits, G. F., 1988. Cardiac growth in rainbow trout, *Salmo gairdneri*. Can. J. Zool. 66(11), 2368-2373. https://doi.org/10.1139/z88-351

Farrell, A. P., Eliason, E. J., Sandblom, E., Clark, T. D., 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. 87(10), 835-851. https://doi.org/10.1139/Z09-092

Faught, E., Aluru, N., Vijayan, M. M., 2016. The Molecular Stress Response. In: Schrek, C. B., Tort, L., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 35, pp. 113–166. Elsevier. https://doi.org/10.1016/B978-0-12-802728-8.00004-7

Fox, J., Weisberg, S., 2019. *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Franklin, C. E., Farrell, A. P., Altimiras, J., Axelsson, M., 2013. Thermal dependence of cardiac function in arctic fish: Implications of a warming world. J. Exp. Biol. 216(22), 4251-4255. https://doi.org/10.1242/jeb.087130

Gallant, M. J., LeBlanc, S., MacCormack, T. J., Currie, S., 2017. Physiological responses to a short-term, environmentally realistic, acute heat stress in Atlantic salmon, *Salmo salar*. FACETS. 2(1), 330–341. https://doi.org/10.1139/facets-2016-0053

Gamperl, A. K., Farrell, A. P., 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. J. Exp. Biol. 207(15), 2539-2550. https://doi.org/10.1242/jeb.01057

Gamperl, A. K., Syme, D. A., 2021. Temperature effects on the contractile performance and efficiency of oxidative muscle from a eurythermal versus a stenothermal salmonid. J. Exp. Biol. 224(15), jeb242487. https://doi.org/10.1242/jeb.242487

Gamperl, A. K., Pinder, A., Grant, R., Boutilier, R., 1994a. Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 193(1), 209–232. https://doi.org/10.1242/jeb.193.1.209

Gamperl, A. K., Vijayan, M. M., Boutilier, R. G., 1994b. Experimental control of stress hormone levels in fishes: Techniques and applications. Rev. Fish. Biol. Fisher. 4(2), 215–255. https://doi.org/10.1007/BF00044129

Gamperl, A. K., Swafford, B. L., Rodnick, K. J., 2011. Elevated temperature, per se, does not limit the ability of rainbow trout in increase stroke volume. J. Therm. Biol. 36(1), 7-14. https://doi.org/10.1016/j.jtherbio.2010.08.007

Gamperl, A. K., Ajiboye, O. O., Zanuzzo, F. S., Sandrelli, R. M., Peroni, E. de F. C., Beemelmanns, A., 2020. The impacts of increasing temperature and moderate hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic salmon (*Salmo salar*). Aquaculture. 519, 734874. https://doi.org/10.1016/j.aquaculture.2019.734874

Gamperl, A. K., Thomas, A. L., Syme, D. A., 2022. Can temperature-dependent changes in myocardial contractility explain why fish only increase heart rate when exposed to acute warming? J. Exp. Biol. 225(4), jeb243152. https://doi.org/10.1242/jeb.243152

Gerber, L., Clow, K. A., Gamperl, A. K., 2020a. Acclimation to warm temperatures has important implications for mitochondrial function in Atlantic salmon (*Salmo salar*). J. Exp. Biol. 224, jeb236257. https://doi.org/10.1242/jeb.236257

Gerber, L., Clow, K. A., Mark, F. C., Gamperl, A. K., 2020b. Improved mitochondrial function in salmon (*Salmo salar*) following high temperature acclimation suggests that there are cracks in the proverbial 'ceiling'. Sci. Rep. 10(1), 21636. https://doi.org/10.1038/s41598-020-78519-4

Gerber, L., MacSween, C. E., Staples, J. F., Gamperl, A. K., Chen, T-Y., 2022. Cold-induced metabolic depression in cunner (*Tautogolabrus adspersus):* A multifaceted cellular event. PLoS ONE. 17(8), e0271086. https://doi.org/10.1371/journal.pone.0271086

Gilbert, M. J. H., Farrell, A. P., 2021. The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic char (Salvelinus alpinus), a northern cold-water specialist. J. Therm. Biol. 95,102816. https://doi.org/10.1016/j.jtherbio.2020.102816

Gilbert, M. J. H., Middleton, E. K., Kanayok, K., Harris, L. N., Moore, J-S., Farrell, A. P., Speers-Roesch, B., 2022. Rapid cardiac thermal acclimation in wild anadromous Arctic char (*Salvelinus alpinus*). J. Exp. Biol. 225(17), jeb244055. https://doi.org/10.1242/jeb.244055

Gollock, M. J., Currie, S., Petersen, L. H., Gamperl, A. K., 2006. Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. J. Exp. Biol. 209(15), 2961-2970. https://doi.org/10.1242/jeb.02319

Graham, M. S., Farrell, A. P., 1989. The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. Physiol. Zool. 62(1), 38–61. https://doi.org/10.1086/physzool.62.1.30159997

Graham, M. S., Farrell, A. P., 1992. Environmental influences on cardiovascular variables in rainbow trout *Oncorhynchus mykiss*, Walbaum. J. Fish. Biol. 41, 851-858. https://doi.org/10.1111/j.1095-8649.1992.tb02713.x

Guderley, H., 2004. Metabolic responses to low temperatures in fish muscle. Biol. Rev. 79(2), 409-427. https://doi.org/10.1017/S1464793103006328

Guderley, H., St. Pierre, J., 1999. Seasonal cycles of mitochondrial ADP sensitivity and oxidative capacities in trout oxidative muscle. J. Comp. Physiol. B. 169, 474-480. https://doi.org/10.1008/s003600050244

Guo, H., Dixon, B., 2021. Understanding acute stress-mediated immunity in teleost fish. Fish. Shellfish. Imun. 2, 100010. https://doi.org/10.1016/j.fsirep.2021.100010

Handeland, S. O., Björsson, B. Th., Arnesen, A. M., Stefansson, S. O., 2003. Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. Aquaculture. 220(1-4), 367-384. https://doi.org/10.1016/S0044-8486(02)00508-2

Handeland, S. O., Imsland, A. K., Stefansson, S. O., 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture. 283(1-4), 36-42. https://doi.org/10.1016/j.aquaculture.2008.06.042

Harter, T. S., Zanuzzo, F. S., Supuran, C. T., Gamperl, A. K., Brauner, C. J., 2019. Functional support for a novel mechanism that enhances tissue oxygen extraction in teleost fish. Proc. R. Soc. B. 286(1903), 20190339. https://doi.org/ 10.1098/rspb.2019.0339

Haverinen, J., Vornanen, M., 2007. Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. Am. J. Phys. Reg. I. 292(2), R1023-R1032. https://doi.org/ 10.1152/ajpregu.00432.2006

Haverinen, J., Vornanen, M., 2020. Reduce ventricular excitability causes atrioventricular block and depression of heart rate in fish at critically high temperatures. J. Exp. Biol. 223, jeb225227. https://doi.org/10.1242/jeb.225227

Holeton, G. F., 1970. Oxygen uptake and circulation by a hemoglobinless antarctic fish (*Chaenocephalus aceratus Lonnberg*) compared with three red-blooded antartic fish. Comp. Biol. Physiol. 34(2), 457–471. https://doi.org/10.1016/0010-406X(70)90185-4

Horodysky, A. Z., Cooke, S. J., Brill, R. W., 2015. Physiology in the service of fisheries science: Why thinking mechanistically matters. Rev. Fish. Biol. Fisheries. 25(3), 425-447. https://doi.org/10.1007/s11160-015-9393-y

Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2017. The effect of thermal acclimation on aerobic scope and critical swim speed in Atlantic salmon (*Salmo salar*). J. Exp. Biol. 220(15), 2757-2764. https://doi.org/10.1242/jeb.154021

Iftikar, F. I., Hickey, A. J. R., 2013. Do mitochondria limit hot fish? Understanding the role of mitochondrial function with heart stress in *Notolabrus celidotus*. PLoS. ONE. 8(5), e64120. https://doi.org/10.1371/journal.pone.0064120

Ignatz, E. H., Sandrelli, R. M., Tibbetts, S. M., Colombo, S. M., Zanuzzo, F. S., Loveless, A. M., Parrish, C. C., Rise, M. L., Gamperl, A. K., 2022. Influence of supplemental dietary cholesterol on growth performance, indices of stress, fillet pigmentation, and upper thermal tolerance of female triploid Atlantic salmon (*Salmo salar*). Aquacult. Nutr. 2022, 1-18. https://doi.org/10.1155/2022/6336060

IPCC, 2018: Summary for Policymakers. In: *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (Eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 3-24, https://doi.org/10.1017/9781009157940.001.

IPCC, 2022: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., https://doi.org/10.1017/9781009325844.

Johansen, I. B., Sandblom, E., Skov, P. V., Gräns, A., Ekström, A., Lunde, I. G., Vindas, M. A., Zhang, L., Höglund, E., Frisk, M., Sjaastad, I., Nilsson, G. E., Øverli, Ø., 2017. Bigger is not better: cortisol-induced cardiac growth and dysfunction in salmonids. 220(14), 2545. https://doi.org/10.1242/jeb.135046

Johnston, I. A., Fleming, J. D., Crockford, T., 1990. Thermal acclimation and muscle contractile properties in cyprinid fish. Am. J. Physiol-Reg. I. 259(2), R231-R236. https://doi.org/10.1152/ajpregu.1990.259.2.R231

Jones, P. L., Sidell, B. D., 1982. Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. II. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. J. Exp. Zool. 219(2), 163-171. https://doi.org/10.1002/jez.1402190205

Jonsson, B., Forseth, T., Jensen, A. J., Naesje, T. F., 2001. Thermal performance of juvenile Atlantic salmon, *Salmo salar* L. Funct. Ecol. 15(6), 701-711. https://doi.org/10.1046/j.0269-8463.2001.00572.x

Keen, J. E., Farrell, A. P., 1994. Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, *Oncorhynchus mykiss,* acclimated to two different water temperatures. Comp. Biochem. Physiol. 108(2-3), 287-295. https://doi.org/10.1016/0300-9629(94)90097-3

Keen, J. E., Gamperl, A. K., 2012. Blood oxygenation and cardiorespiratory function in steelhead trout (*Oncorhynchus mykiss*) challenged with an acute temperature increase and zatebradine-induce bradycardia. J. Therm. Biol. 37(3), 201-210. https://doi.org/10.1016/j.jtherbio.2012.01.002

Kent, J., Koban, M., Prosser, C. L., 1988. Cold-acclimation-induced protein hypertrophy in channel catfish and green sunfish. J. Comp. Physiol. B. 158(2), 185–198. https://doi.org/10.1007/BF01075832

Killen, S. S., Andriaenssens, B., Marras, S., Claireaux, G., Cooke, S. J., 2016. Context dependency of trait repeatability and its relevance for management and conservation of fish populations. Conserv. Physiol. 4(1), cow007. https://doi.org/10.1093/conphys/cow007

Killen, S. S., Christensen, E. A. F., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A., Munson, A., Nati, J. J. H., Papatheodoulou, M., McKenzie, D. J., 2021. Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. J. Exp. Biol. 224(18), jeb242522. https://doi.org/10.1242/jeb.242522

Klaiman, J. M., Fenna, A. J., Shiels, H. A., Macri, J., Gillis, T. E., 2011. Cardiac remodeling in fish: Strategies to maintain heart function during temperature change. Plos. One. 6(9), e24464. https://doi.org/10.1371/journal.pone.0024464

Kline, R., Parkyn, D., & Murie, D., 2015. Empirical modelling of solid-blocking effect in a Blazka respirometer for gag, a large demersal reef fish. Adv. Zool. Bot. 3, 193–202. https://doi.org/10.13189/azb.2015.030501

Komsta, L., 2022. *Outliers:* Test for outliers. R package version 0.15. http://CRAN.R-project.org/package=outlier

Kuznetsova, A., Brockhoff, P. B., Christensen, R. H. B., 2017. *lmerTest Package*: Tests in Linear Mixed Effects Models. J. Stats. Softw. 82(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Lannig, G., Bock, C., Sartoris, F. J., Pörtner, H. O., 2004. Oxygen limitation of thermal tolerance in cod (*Gadus morhua*) studied by magnetic resonance imaging and on-line venous oxygen monitoring. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287(4). R902-R910. https://doi.org/10.1152/ajpregu.00700.2003

Leeuwis, R. H. J., Nash, G. W., Sandrelli, R. M., Zanuzzo, F. S., Gamperl, A. K., 2019. The environmental tolerances and metabolic physiology of sablefish (*Anoplopoma fimbria*). Comp. Biochem. Physiol. A. 231, 140-148. https://doi.org/ 10.1016/j.cbpa.2019.02.004

Leeuwis, R. H. J., Zanuzzo, F. S., Peroni, E. F. C., Gamperl, A. K., 2021. Research on sablefish (*Anoplopoma fimbria*) suggests that limited capacity to increase heart function leaves hypoxic fish susceptible to heat waves. Proc. R. Soc. B. 288(1946), 20202340. https://doi.org/10.1098/rspb.2020.2340

Little, A. G., Loughland, I., Seebacher, F., 2020. What do warming waters mean for fish physiology and fisheries? J. Fish. Biol. 97(2), 328-340. https://doi.org/10.1111/jfb.14402

Maule, A. G., Schreck, C. B., Kaattari, S. L., 1987. Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during parr-to-smolt transformation and after implantation of cortisol. Can. J. Fish. Aquat. Sci. 44(1), 161-166. https://doi.org/10.1139/f87-021

McArley, T. J., Morgenroth, D., Zena, L. A., Ekström, A. T., Sandblom, E., 2021. Normoxic limitation of maximal oxygen consumption rate, aerobic scope and cardiac performance in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 224(15), jeb242614. https://doi.org/10.1242/jeb.242614

McArley, T. J., Morgenroth, D., Zena, L. A., Ekström, A. T., Sandblom, E., 2022. Prevalence and mechanisms of environmental hyperoxia-induced thermal tolerance. Proc. R. Soc. B. 289(1981), 20220840. https://doi.org/ 10.1098/rspb.2022.0840

Millard, S. P., 2013. *EnvStats:* An R Package for Environmental Statistics. Springer, New York. ISBN 978-1-4614-8455-4, https://www.springer.com.

Morgan, R., Andreassen, A. H., Åsheim, E. R., Jutfelt, F., 2022. Reduce physiological plasticity in a fish adapted to stable temperatures. Proc. Natl. Acad. Sci. 119(22), e2201919119. https://doi.org/10.1073/pnas.2201919119

Muñoz, N. J., Farrell, A. P., Heath, J. W., Neff, B. D., 2018. Hematocrit is associated with thermal tolerance and modulated by developmental temperature in juvenile chinook salmon. Physiol. Biochem. Zool. 91(1), 757–762. https://doi.org/10.1086/695556

Norin, T., Canada, P., Baily, J. A., Gamperl, A. K., 2019. Thermal biology and swimming performance of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). PeerJ. 7, e7784. https://doi.org/10.7717/peerj.7784

O'Brien, K. M., 2011. Mitochondrial biogenesis in cold-bodied fishes. J. Exp. Biol. 214(2), 275-285. https://doi.org/10.1242/jeb.046854

Pearson, M. P., Stevens, E. D., 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish. Physiol. Biochem. 9(1), 39–50. https://doi.org/10.1007/BF01987610

Pickering, A. D., Pottinger, T. G., 1989. Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. Fish. Physiol. Biochem. 7(1-6), 253-258. https://doi.org/10.1007/BF00004714

Porter, E. S., Clow, K. A., Sandrelli, R. M., Gamperl, A. K., 2022. Acute and chronic cold exposure differentially affect cardiac control, but not cardiorespiratory function, in resting Atlantic salmon (*Salmo salar*). Curr. Res. Phys. 5, 158-170. https://doi.org/10.1016/j.crphys.2022.03.002

Pörtner, H. O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften. 88(4), 137-146. https://doi.org/10.1007/s001140100216.

Pörtner, H. O., 2010. Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213(6), 881–893. https://doi.org/10.1242/jeb.037523

Pörtner, H. O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science. 315(5808), 95–97. https://doi.org/10.1126/science.1135471

R Core Team, 2022. R: A lamguage and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Reid, C. H., Patrick, P. H., Rytwinski, T., Taylor, J. J., Willmore, W. G., Reesor, B., Cooke, S. J., 2022. An updated review of cold shock and cold stress in fish. J. Fish. Biol. 100(5), 1102-1137. https://doi.org/10.1111/jfb.15037

Riseth, E. N., Fraser, T. W. K., Sambraus, F., Stien, L. H., Hvas, M., 2020. Is it advantageous for Atlantic salmon to be triploid at lower temperatures? J. Therm. Biol. 89, 102548. https://doi.org/10.1016/j.jtherbio.2020.102548

Rodgers, G. G., Tenzing, P., Clark, T. D., 2016. Experimental methods in aquatic respirometry: The importance of mixing devices and accounting for background respiration: methods in aquatic respirometry. J. Fish. Biol. 88(1), 65–80. https://doi.org/10.1111/jfb.12848

Rodnick, K. J., Planas, J. V., 2016. The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments. In: Shreck, C. B., Tort, L., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 35, pp. 251-294. Elsevier. https://doi.org/10.1016/B978-0-12-802728-8.00007-2

Rome, L. C., Swank, D. M., 2001. The influence of thermal acclimation on power production during swimming I. *In Vivo* stimulation and length change pattern of scup red muscle. J. Exp. Biol. 204(3), 409-418. https://doi.org/10.1242/jeb.204.3.409

Rome, L. C., Loughna, P. T., Goldspink, G., 1985. Temperature acclimation: Improved sustained swimming in carp at low temperatures. Science. 228(4696), 194-196. https://doi.org/10.1126/science.228.4696.194

Rome, L. C., Sosnicki, A., Choi, I. H., 1992. The influence of temperature on muscle function in the fast swimming sculp. II. The mechanics of red muscle. J. Exp. Biol. 163(1), 281-295. https://doi.org/10.1242/jeb.163.1.281

Ruud, J. T., 1954. Vertebrates without erythrocytes and blood pigment. Nature. 173(4410), 848–850. https://doi.org/10.1038/173848a0

Sandblom, E., Axelsson, M., 2007. Venous hemodynamic responses to acute temperature increase in the rainbow trout (*Oncorhynchus mykiss*). Am. J. Physiol-Reg. I. 292(6), 2292-2298. https://doi.org/10.1152/ajpregu.00884.2006

Sandblom, E., Gräns, A., Axelsson, M., Seth, H., 2014. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: Implications in a thermally extreme future. Proc. Royal. Soc. B. 281(1794), 20141490. https://doi.org/10.1098/rspb.2014.1490

Shreck, C. B., 2000. Accumulation and Long-term Effects of Stress in Fish. In: Moberg, G. P., Mench, J. A., (Eds.). The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare, pp. 147-158. CABI. https://doi.org/10.1079/9780851993591.0147

Schreck, C. B., Tort, L., 2016. The Concept of Stress in Fish. In: Shreck, C. B., Tort, L., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 35, pp. 1–34. Elsevier. https://doi.org/10.1016/B978-0-12-802728-8.00001-1

Shuman, J. L., Coughlin, D. J., 2018. Red muscle function and thermal acclimation to cold in rainbow smelt, *Osmerus mordax*, and rainbow trout, *Oncorhynchus mykiss*. J. Exp. B. 329(10), 547-556. https://doi.org/10.1002/jez.2219

Smith, L. S., Bell, G. R., 1964. A technique for prolonged blood sampling in free-swimming salmon. J. Fish. Res. Board. Can. 21(4), 711–717. https://doi.org/10.1139/f64-064

Steinhausen, M. F., Sandblom, E., Eliason, E. J., Verhille, C., Farrell, A. P., 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). J. Exp. Biol. 211(24), 3915-3926. https://doi.org/10.1242/jeb.019281

Sutcliffe, R. L., Li, S., Gilbert, M. J. H., Schulte, P. M., Miller, K. M., Farrell, A. P., 2020. A rapid intrinsic heart rate resetting with thermal acclimation in rainbow trout, *Oncorhynchus mykiss*. 223, jeb215210. https://doi.org/10.1242/jeb.215210

Svendsen, M. B. S., Bushnell, P. G., Steffensen, J. F., 2016. Design and setup of intermittent-flow respirometry system for aquatic organisms: How to set up an aquatic respirometry system. J. Fish. Biol. 88(1), 26–50. https://doi.org/10.1111/jfb.12797

Szekeres, P., Eliason, E., Lapointe, D., Donaldson, M., Brownscombe, J., Cooke, S., 2016. On the neglected cold side of climate change and what it means to fish. Clim. Res. 69(3), 239–245. https://doi.org/10.3354/cr01404

Tanck, M. W. T., Booms, G. H. R., Eding, E. H., Bonga, S. E. W., Komen, J., 2000. Cold shocks: A stressor for common carp. J. Fish. Biol. 57(4), 881–894. https://doi.org/10.1111/j.1095-8649.2000.tb02199.x

Taugbøl, A., Olstad, K., Bærum, K. M., Museth, J., 2019. Swimming performance of brown trout and grayling show species-specific responses to changes in temperature. Ecol. Freshw. Fish. 28(2), 241-246. https://doi.org/10.1111/eff.12447

Taylor, E. B., McPhail, J. D., 1985. Variation in burst and prolonged swimming performance among British Columbia populations of Coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42(12), 2029–2033. https://doi.org/10.1139/f85-250

Taylor, E. S., Egginton, S., Taylor, E. W., 1996. Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. J. Exp. Biol. 199(4), 835-845. https://doi.org/10.1242/jeb.199.4.835

Taylor, E. W., Egginton, S., Taylor, S. E., Butler, P. J., 1997. Factors which may limit swimming performance at different temperatures. In: Wood, C. M., McDonald, C. G., (Eds.). Global Warming Implications for Freshwater and Marine Fish, pp. 105-133. Cambridge University Press.

USGCRP, 2017. *Climate Science Special Report: Fourth National Climate Assessment, Volume I.* Wuebbles, D. J., Fahey, D. W., Hibbard, K. A., Dokken, D. J., Stewart, B. C., Maycock, T. K., (Eds.). U.S. Global Change Research Program, Washinton, DC, USA, pp. 470. https://doi.org/10.7930/J0J964J6

Vadboncoeur, E., Nelson, C., Hall, J.R., Clow, K.A., Sandrelli, R.M., Brauner, C.J., Swanson, A.K., Gamperl, A.K., 2023. Lowering temperature to 1˚C results in physiological changes, stress and mortality in cultured Atlantic salmon (*Salmo salar*). Aquaculture. https://doi.org/10.1016/j.aquaculture.2023.739313

Vornanen, M., 1998. L-type Ca^{2+} current in fish cardiac myocytes: effects of thermal acclimation and beta-adrenergic stimulation. J. Exp. Biol. 201(4), 533-547. https://doi.org/10.1242/jeb.201.4.533

Vornanen, M., 2016. The temperature dependence of electrical excitability in fish hearts. J. Exp. Biol. 219(13), 1941-1952. https://doi.org/10.1242/jeb.128439

Vornanen, M., 2020. Feeling the heat: source-sink mismatch as a mechanism underlying the failure of thermal tolerance. J. Exp. Biol. 223(16), jeb225680. https://doi.org/10.1242/jeb.225680

Vornanen, M., Ryökkynen, A., Nurmi, A., 2002a. Temperature-dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. Am. J. Phys. Reg. I. 282(4), R1191-R1199. https://doi.org/10.1152/ajpregu.00349.2001

Vornanen, M., Shiels, H., Farrell, A. P., 2002b. Plasticity of excitation-contraction coupling in fish cardiac myocytes. Comp. Biochem. Phys. A. 132(4), 827-846. https://doi.org/10.1016/S1095-6433(02)00051-X

Wang, T., Overgaard, J., 2007. Ecology: The heartbreak of adapting to global warming. Science. 315(5808), 49–50. https://doi.org/10.1126/science.1137359

Yada, T., Tort, L., 2016. Stress and Disease Resistance: Immune System and Immunoendocrine Interactions. In: Schreck, C. B., Tort, L., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 35, pp. 365-403. Elsevier Academic Press. https://doi.org/10.1016/B978-0-12-802728-8.00010-2

Zanuzzo, F. S., Bailey, J. A., Garber, A. F., Gamperl, A. K., 2019. The acute and incremental thermal tolerance of Atlantic cod (*Gadus morhua*) families under normoxia and mild hypoxia. Comp. Biochem. Physiol. A. 233, 30–38. https://doi.org/10.1016/j.cbpa.2019.03.020

CHAPTER 4: Summary and Perspectives

4.1 General Discussion

This thesis is not the first to investigate the physiology of temperate fishes, including Atlantic salmon (*Salmo salar*), in the era of climate change (Alfonso et al., 2021; Antilla et al., 2013, 2014; Beemelmanns et al., 2021; Ekström et al., 2019; Farrell et al., 2009; Gamperl et al., 2019, 2020; Gerber et al., 2020; Hvas et al., 2017; Sutcliffe et al., 2020; Tromp et al., 2018). However, while there have been considerable efforts to predict what will happen to teleost fish in a warming climate, the effects of extremely cold/low temperatures on salmon physiology are still relatively unknown. Therefore, the purpose of this thesis was to better understand the impacts of acute and chronic cold events, similar to winter temperatures and 'super-chill' events observed at coastal sea-cages in Atlantic Canada, Iceland and Norway (Reid et al., 2022; Szekeres et al., 2016) on the salmon's cardiorespiratory and stress physiology, and swimming capacity. By examining aspects of cardiac function and control, metabolism, the stress response and the swimming capacity of cultured Atlantic salmon (*Salmo salar*) acclimated to 8, 4 and 1°C, and when acutely cooled from 8 to 1°C, this work highlights: 1) how the effects of short- vs. long-term exposure to sublethal low temperatures differ; and 2) whether these fish are phenotypically 'plastic' and can respond appropriately to such environmental changes. Overall, it is hoped that the information contained in this thesis, and future intra- and inter-specific comparisons of fish physiology when faced with temperatures near their lower thermal limits, will allow fisheries managers, conservationists, and aquaculture companies to better predict the implications of minimum winter temperatures and 'super-chill' events on coastal fishes and aquaculture production.

4.2 Novel Findings

4.2.1 Salmon acclimation capacity at cold temperatures

It remains unclear how, or if, salmon held in aquaculture cages can adapt to extremely cold temperatures (as they cannot migrate to warmer waters), which is currently an issue faced by thousands of salmon held in commercial sea-cages and has been linked to mortalities (Evans 2019; Huffman 2019). As expected, the results from Chapters 2 and 3 demonstrate that exposure to low temperatures, whether over hours or weeks, significantly reduces the routine metabolism and resting cardiac function of salmon. However, they also show that the cardiorespiratory response to cold temperatures differed between the two studies. All fish tested at 1°C in Chapter 2, regardless of acclimation temperature, had comparable values of intrinsic and resting f_H (~ 22 and 32 beats min^{-1}, respectively), and the latter was extremely similar to resting values observed in Chapter 3 for 1°C acclimated fish (~ 34 beats min^{-1}). However, the acutely exposed fish in Chapter 3 had a significantly lower resting f_H (27.6 ± 1.0 beats min^{-1}). In addition, the capacity for cardiac remodelling differed between the experiments, with fish in Chapter 2 showing no change in their relative ventricular mass (RVM) when acclimated to 1°C, while the RVM in fish from Chapter 3 increased 1.17 and 1.19 times with cold acclimation (at 4 and 1°C, respectively). Overall, the differences in resting f_H and cardiac hypertrophy (larger RVM) between the two experiments were unforeseen and indicate that intraspecific differences exist in the phenotypic responses of salmon to temperatures close to their lower thermal limit and during 'cold shocks'. This is likely to not only be between cultured stocks, but also between cultured and wild stocks. Clearly, the effects of acclimation, and acute exposure, to such cold temperature on the cardiorespiratory physiology of wild Atlantic salmon should also be investigated.

The potential for plasticity, and the time-course required to elicit phenotypic responses is not clear based on current studies of cold-acclimation (Lurman et al., 2012; Sutcliffe et al., 2020). For example, as discussed in Chapter 2, temperature coefficient (Q_{10}) values should be lower for fish acclimated to a particular thermal environment when compared to those for fish acutely exposed to the same temperature. In Chapter 2 it was concluded that salmon either: reset their f_H quickly as temperature dropped; or that these fish were not able to physiologically compensate/acclimate to 1°C even after 12 weeks [i.e., their f_H was the same as conspecifics acutely exposed to this temperature over 8 h and had similar Q_{10} values (~2.5-3)]. While the latter raises the possibility that phenotypic plasticity is too "biologically costly" for cultured salmon, it does not appear to apply to the salmon used in Chapter 3 (i.e., from a genetically distinct population). Resting cardiorespiratory parameters in these salmon did exhibit a compensatory thermal response to being acclimated to 4 and 1°C when compared to those acclimated to 8°C and acutely exposed to 1°C. More specifically, the Q_{10} values for standard metabolic rate (SMR) and resting f_H in salmon acclimated to 1°C were lower (1.16 and 2.48, respectively) than fish acutely exposed (2.10 and 3.44, respectively) to the same temperature.

The idea that the plastic response(s) of several physiological traits associated with acclimation have been lost in some cultured salmon is not far-fetched. For example, it was recently shown that zebrafish bred/held at a constant temperature in a lab-based setting completely lost their ability to cope (via changes in metabolism, thermal tolerance, and gene expression) when challenged with a change in temperature compared to wild populations (Morgan et al., 2022). Therefore, it is possible that the population of salmon used in Chapter 3 were better selected for performance/survival when exposed to changes in their thermal environment compared to those in Chapter 2 given that: 1) the salmon in Chapter 3 were derived from stocks that are reared in coastal

sea-cages, and therefore exposed to varying ocean conditions during the grow out phase; 2) that salmon from Chapter 2 had been reared entirely in land-based systems where water temperatures are controlled and relatively constant (~6 -15°C, the latter optimal for growth; Ignatz et al., 2022) throughout their life history, and have been for several generations (i.e., over approximately the past 25 years); and most convincing 3) that fish used in Chapter 2 failed to demonstrate an acclimation response whereas such a response was observed in the salmon used in Chapter 3. Although no genetic testing was completed as part of this research, the hypothesis that Atlantic salmon reared in sea-cages have a greater capacity for phenotypic plasticity is also supported by the recent work of Adams et al. (2022). These authors showed that 50 years of genetic selection for the Australian H-strain rainbow trout has resulted in a population with an unusually high and broad thermal acclimation window. Further, studies conducted on different strains/families of rainbow trout and Atlantic salmon have concluded that there is significant intraspecific variation in phenotypic traits related to both temperature and hypoxia tolerance, and that these differences are reflected in their aerobic potential, CT_{max} and P_{crit} (Antilla et al., 2013; Zhang et al., 2018). Collectively, these data suggest that intraspecific variation plays a crucial role in how different populations of cultured fishes respond to, and tolerate, temperature changes. This information should be highly considered in selecting breeding programs for Atlantic salmon sea-cage production as these fish will inevitably experience even more drastic flucatuations in temperature (both high and low) than the current recordings due to accelerated climate change.

4.2.2 Maximum performance at cold temperatures

The effects of cold temperatures on salmon maximal swimming and cardiorespiratory performance were reported and discussed in detail in Chapter 3. Although acclimation to 1°C

resulted in a higher resting f_H compared to acutely cooled fish, there were no differences in the maximum f_H achieved when tested at 1°C (**Table S6**); these data suggesting that an increase in resting f_H during acclimation is not reflected in a higher maximum f_H. In addition, while fish acclimated to 1°C had a significantly higher resting tissue oxygen extraction (estimated as $\dot{M}O_2 / \dot{Q}$) and a large scope to increase stroke volume (V_S) when exercised (i.e., to meet tissue oxygen demands), the opposite was true for fish acutely exposed to 1°C (which had a high resting V_S and a large scope for $\dot{M}O_2 / \dot{Q}$). Although it was expected that V_S would contribute more to the increase in $\dot{Q}$ when the salmon were exercised at cold temperatures (Gamperl et al., 2011, 2022), it is still not clear from this work which cardiovascular/hemodynamic trait(s) are responsible for the modulation of V_S under these conditions. If the capacity for salmon to increase f_H is limited regardless of the time spent at 1°C (and thus, the diastolic period would be expected to be similar between groups), and an increase in RVM (as seen in acclimated fish) does not facilitate a higher maximum V_S, then the regulation of central venous pressure (CVP) is likely a significant factor in determining their maximum Vs and cardiac performance (Brijs et al., 2017). Collectively, these results show that salmon can modulate maximum cardiovascular function/cardiorespiratory capacity via different mechanisms when acutely vs. chronically exposed to cold temperatures. However, why f_H doesn't play a major role in increasing cardiac output ($\dot{Q}$) in these environments remains to be determined. It is possible that myocardial twitch kinetics are hindered at cold temperatures (Gamperl et al., 2022). However, it is also likely that the cardiomyocyte's electrophysiology and/or the function/density of important ion channels in the heart contribute to the observed differences (Haverinen and Vornanen, 2007, 2020; Vornanen 2016, 2017; Vornanen et al., 2002). Lastly, while it is clear that there is considerable plasticity in how salmon meet tissue oxygen demands (i.e., by increasing either V_S or $\dot{M}O_2 / \dot{Q}$) at cold temperatures, which parameter

is modulated depends on the length of cold exposure, and it is unknown why acclimation to 1°C impaired the swimming capacity of salmon more than an acute drop in temperature. Interestingly, both groups had similar values of maximum metabolic rate (MMR) and absolute aerobic scope (AS) (**Table S6**), indicating that differences in aerobic capacity did not contribute to the reduced U_{crit} in salmon acclimated vs. acutely exposed to cold temperatures. Therefore, it is likely that physiological and/or anatomical limits within the red muscle itself were probably constraining the swimming performance of 1°C acclimated fish (Crockford and Johnson, 1992; Egginton and Cordiner, 1997; Guderley and St. Pierre, 1999; Klaiman et a., 2014; Rome et al., 1992; Taylor et al., 1996).

4.3 Future Research

4.5.1 Cardiorespiratory physiology

Given that the intrinsic f_H of both groups of salmon tested at 1°C in Chapter 2 were extremely similar, it is likely that the effects of cold exposure on the cardiac pacemaker cells were comparable regardless of time spent at this temperature. Intrinsic f_H was not examined in Chapter 3, and therefore, I cannot conclude that acclimation to 1ºC was able to fully reset the salmon's pacemaker function. Thus, future experiments should examine the effects of short- and long-term cold exposure on the pacemaker cells of phenotypically plastic strains of salmon via patch-clamp techniques to investigate how the duration of cold exposure affects the density and function of important ion channels/currents that are responsible for initiating contraction of the heart (as described for rainbow trout pacemaker cells by Haverinen and Vornanen, 2007). Further, we should aim to better understand the effects of temperature on the spread of electrical excitation propagated from the pacemaker cells and across the atrium and ventricle (Lin et al, 2015; Stoyek

et al., 2016). A delay at the atrioventricular (AV) junction allows for sufficient filling of the ventricle between contractions (as reviewed in Vornanen 2017). Therefore, it would be interesting to determine if this delay, or the conductance velocity of excitation across these chambers, is directly impacted by changes in temperature. Further, *in situ* heart experiments (e.g. Lurman et al., 2012), and those using isolated cycling muscle strips (e.g. Gamperl and Syme, 2021, Gamperl et al., 2022) could be used to examine whether cold acclimation, and/or the elevated cortisol levels associated with it, diminish the hearts pumping capacity, and whether this is directly related to characteristics of myocardial performance. Ultimately, research on isolated hearts and preparations can help answer the question: what determines maximum f_H and the heart's pumping capacity between and within species across their thermal niche.

The modulation of V_S and role of $\dot{M}O_2 / \dot{Q}$ in meeting tissue oxygen demands also needs to be examined further. Future research should explore why salmon at cold temperatures do not increase both parameters when challenged with increased cardiac and energetic demands. For example, it is possible that prolonged cold exposure negatively influences a salmon's ability to control/increase CVP while swimming. This research question could be addressed by inserting a catheter into the Ductus Cuvier (DC; a major vein which drains into the sinus venosus) and measuring central venous pressure (Altimiras and Axelsson, 2004; Minerick et al., 2003; Olson et al., 1997) using a pressure transducer while salmon are resting and while they perform a U_{crit} test. In addition, blood could be withdrawn simultaneously from the DC and dorsal aortic cannulae, and this would allow for the simultaneous measurement of arterial and venous blood oxygen content. Such measurements would hopefully confirm that my interpretation (based $\dot{M}O_2 / \dot{Q}$) of how acute vs. long-term cold exposure affect tissue oxygen extraction is correct/accurate. These experiments would not be easy to perform. However, such studies would provide important, and

potentially novel, information on the control of cardiac function and cardiorespiratory plasticity in fish at cold temperatures.

4.3.2 Red muscle function and energetics

While the collected data provide few insights into why fish acclimated to 1°C had a lower maximum swim speed (U_{crit}) than the acutely cooled fish, it could be hypothesized that red muscle recruitment and/or performance (i.e., power production, endurance, aerobicity) in the former group were negatively affected by chronic exposure to cold temperatures. The recruitment of red muscle is required for sustained aerobic activity, with the fish's white muscle only used significantly for fast/short locomotory activities or when fish such as salmonids reach ~ 70-80% of their U_{crit} [i.e., when they begin burst and coast swimming (Burgetz et al., 1998; Jones, 1982; Sisson and Sidell, 1987)]. There is evidence that cold acclimation can induce earlier recruitment of white muscle (Rome et al., 1984), and this may be because the function of the red muscle has been diminished or is fully recruited at lower swimming speeds. In this regard, the implantation of EMG electrodes and/or sonomicrometry crystals into the red and white muscle (see Beddow and McKinley, 1999; Cooke et al., 2004; Coughlin et al., 1996; Gollock et al., 2009; Taylor et al., 1996) would be very useful. These techniques would allow for the timing and extent of the use of these two muscle types to be examined when fish are chronically and acutely exposed to 1°C. Such experiments could be followed by cycling muscle preparations (strips) where red muscle contraction kinetics (Syme et al., 2008), performance (e.g., power output, duration of contraction and relaxation) and efficiency could be measured at contraction rates and stains that mimic *in vivo* conditions (e.g., as in Gamperl and Syme 2021), and histological analyses could be used to examine whether a

reduction in red muscle capillary density may constrain the performance capacity of 1ºC acclimated fish (Johnston, 1982).

4.3.3 A global perspective

The research conducted in this thesis, and that by my colleague Émile Vadboncoeur (Vadboncoeur et al., in final prep), is amongst the first to comprehensively examine which physiological mechanisms influence/determine salmon survival at extremely cold temperatures and may be applicable in a larger geographic context. For example, similar physiological constraints and/or phenotypic plasticity (or lack thereof) may be observed in species from tropical or subtropical coastal regions. Although suboptimal temperatures haven't been a primary interest of the fish physiology research community [with the exception of research conducted on Antarctic fish and other polar species (Farrell and Steffensen, 2005)], these marine environments (incl. patch reefs, mangrove forests, and other nearshore ecosystems) are projected to experience some of the harshest impacts of climate change (IPCC 2018; 2022) and are currently inhabited by many key species that have both economic and ecological significance. Although 'cold' temperatures at these latitudes are much higher than those for salmon in the North Atlantic [i.e., 15-25℃ vs. 0-3℃ (Björnsson et al., 2007; Giomi et al., 2019; Holm 2000; Nowell et al., 2015)], the absolute difference in temperatures they experience is relatively similar (i.e., a 15-20℃ range in water temperatures over the year). More recent work has also documented large diurnal fluctuations in temperature and water oxygen in such environments (Giomi et al., 2019; Porter et al., unpublished work). It would be interesting to see if warm water fishes which endure similar thermal shifts respond to/control their cardiac function and metabolism in a similar manner to salmon: specifically if they also rely on V_S rather than f_H to increase $\dot{Q}$ near their lower thermal limits, and

if their dependence on V_S vs. $\dot{M}O_2 / \dot{Q}$ is influenced by their duration of exposure to these temperatures. By conducting such studies, we will be better equipped to understand which fishes may/will be more susceptible or resilient to climate change on a global scale.

4.4 References

Adams, O. A., Zhang, Y., Gilbert, M. H., Lawrence, C. S., Snow, M., Farrell, A. P., Cooke, S., 2022. An unusually high upper thermal acclimation potential for rainbow trout. Conserv. Biol. 10(1), coab101. https://doi.org/10.1093/conphys/coab101

Alfonso, S., Gesto, M., Sadoul, B., 2021. Temperature increase and its effects on fish stress physiology in the context of global warming. J. Fish. Biol. 98(6), 1496–1508. https://doi.org/10.1111/jfb.14599

Altimiras, J., Axelsson, M., 2004. Intrinsic autoregulation of cardiac output in rainbow trout (*Oncorhynchus mykiss*) at different heart rates. J. Exp. Biol. 207(2), 195-201. https://doi.org/10.1242/jeb.00742

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R., 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. J. Exp. Biol. 216(7), 1183–1190. https://doi.org/10.1242/jeb.080556

Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E., Farrell, A. P., 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. Nat. Commun. 5(1), 4252. https://doi.org/10.1038/ncomms5252

Beddow, T. A., McKinley, R. S., 1999. Importance of electrode positioning in biotelemetry studies estimating muscle activity in fish. J. Fish. Biol. 54(4), 819-831. https://doi.org/ 10.1111/j.1095-8649.1999.tb02035.x

Beemelmanns, A., Ribas, L., Anastasiadi, D., Moraleda-Prados, J., Zanuzzo, F. S., Rise, M. L., Gamperl, A. K., 2021. DNA methylation dynamics in Atlantic salmon (*Salmo salar*) challenged with high temperature and moderate hypoxia. Front. Mar. Sci. 7, 604878. https://doi.org/10.3389/fmars.2020.604878

Björnsson, H., Olason, E. O., Jónsson, T., Henriksen, S., 2007. Analysis of a smooth seasonal cycle with daily resolution and degree day maps for Iceland. Meteorol. Z. 16(1), 57–69. https://doi.org/10.1127/0941-2948/2007/0188

Brijs, J., Sandblom, E., Dekens, E., Näslund, J., Ekström, A., Axelsson, M., 2017. Cardiac remodelling and increased central venous pressure underlie elevated stroke volume and cardiac output of seawater-acclimated rainbow trout. Am. J. Physiol-Reg. I. 312(1), R31-R39. https://doi.org/10.1152/ajpregu.00374.2016

Burgetz, I. J., Rojas-Vargas, A., Hinch, S. G., Randall, D. J., 1998. Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 201(19), 2711-2721. https://doi.org/10.1242/jeb.201.19.2711

Cooke, S. J., Thorstad, E. B., Hinch, S. G., 2004. Activity and energetics of free-swimming fish: insights from electromyogram telemetry. Fish. Fish. 5(1), 21-52. https://doi.org/10.1111/j.1467-2960.2004.00136.x

Coughlin, D. J., Valdes, L., Rome, L. C., 1996. Muscle length changes during swimming in scup: sonomicrometry verifies the anatomical high-speed cine technique. J. Exp. Biol. 199(2), 459-463. https://doi.org/10.1242/jeb.199.2.459

Crockford, T., Johnston, I.A., 1990. Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscle of the common carp (*Cyprinus carpio L.*). J. Comp. Physiol. 160(1), 23-30. https://doi.org/10.1007/BF00258759

Egginton, S., Cordiner, S., 1997. Cold-induced angiogenesis in seasonally acclimatized rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 200(16), 2263-2268. https://doi.org/10.1242/jeb.200.16.2263

Ekström, A., Gräns, A., Sandblom, E., 2019. Can´t beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. J. Comp. Physiol. B. 189(6), 757–769. https://doi.org/10.1007/s00360-019-01243-7

Evans, O. (2019). Cooke loses salmon to superchilled water. Retrieved October 28, 2022 from https://salmonbusiness.com/cooke-loses-salmon-to-superchilled-water/#:~:text=Cooke%20Aquaculture%20reports%20mortalities%20at,so%2Dcalled%20%E2%80%9Csuperchill%E2%80%9D.&text=The%20company%20is%20following%20its%20Farm%20Management%20plan.

Farrell, A. P., Steffensen, J. F., (Eds.) 2005. The Physiology of Polar Fishes. Elsevier Acad. Press, Amsterdam and Heidelberg.

Farrell, A. P., Eliason, E. J., Sandblom, E., Clark, T. D., 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. 87(10), 835–851. https://doi.org/10.1139/Z09-092

Gamperl, A. K., Syme, D. A., 2021. Temperature effects on the contractile performance and efficiency of oxidative muscle from a eurythermal versus a stenothermal salmonid. J. Exp. Biol. 224(15), jeb242487. https://doi.org/10.1242/jeb.242487

Gamperl, A. K., Ajiboye, O. O., Zanuzzo, F. S., Sandrelli, R. M., Peroni, E. de F. C., Beemelmanns, A., 2020. The impacts of increasing temperature and moderate hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic Salmon (*Salmo salar*). Aquaculture. 519, 734874. https://doi.org/10.1016/j.aquaculture.2019.734874

Gamperl, A. K., Swafford, B. L., Rodnick, K.J., 2011. Elevated temperature, per se, does not limit the ability of rainbow trout to increase stroke volume. J.Therm. Biol. 36(1), 7-14. https://doi.org/10.1016/j.jtherbio.2010.08.007

Gamperl, A. K., Thomas, A. L., Syme, D. A., 2022. Can temperature-dependent changes in myocardial contractility explain why fish only increase heart rate when exposed to acute warming? J. Exp. Biol. 225(4), jeb243152. https://doi.org/10.1242/jeb.243152

Gerber, L., Clow, K. A., Gamperl, A. K., 2020a. Acclimation to warm temperatures has important implications for mitochondrial function in Atlantic salmon (*Salmo salar*). J. Exp. Biol. 224, jeb.236257. https://doi.org/10.1242/jeb.236257

Giomi, F., Barausse, A., Booth, J., Agusti, S., Saderne, V., Anton, A., Daffonchio, D., Fusi, M., 2019. Oxygen supersaturation protects coastal marine fauna from ocean warming. Sci. Adv. 5(9), eaax1814. https://doi.org/10.1126/sciadv.aax1814

Gollock, M. J., Syme, D. A., Freeman, M., McKinley. R. S., Gamperl, A. K., 2009. Potential methods for measuring the activity patterns and energy use of Atlantic cod (Gadus morhua). Can. J. Fish. Aquat. Sci. 66(7), 1095-1106. https://doi.org/10.1139/F09-062

Guderley, H., St. Pierre, J., 1999. Seasonal cycles of mitochondrial ADP sensitivity and oxidative capacities in trout oxidative muscle. J. Comp. Physiol. B. 169, 474-480. https://doi.org/10.1008/s003600050244

Haverinen, J., Vornanen, M., 2007. Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. Am. J. Phys. Reg. I. 292(2), R1023-R1032. https://doi.org/ 10.1152/ajpregu.00432.2006

Haverinen, J., Vornanen, M., 2020. Reduce ventricular excitability causes atrioventricular block and depression of heart rate in fish at critically high temperatures. J. Exp. Biol. 223, jeb225227. https://doi.org/10.1242/jeb.225227

Holm, M., Holst, J. C., Hansen, L. P., 2000. Spatial and temporal distribution of post-smolts of Atlantic salmon (*Salmo salar L.*) in the Norwegian Sea and adjacent areas. ICES. J. Mar. Sci. 57(4), 955–964. https://doi.org/10.1006/jmsc.2000.0700

Huffman, J., 2019, March 17. Cooke experiences 'super chill' event off Nova Scotia coast. Retrieved September 25, 2021, from Atlantic Salmon Federation: https://www.asf.ca/news-and-magazine/salmon-news/cooke-experiences-super-chill-

Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2017. The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. J. Exp. Biol. 220(15), 2757–2764. https://doi.org/10.1242/jeb.154021

Ignatz, E. H., Sandrelli, R. M., Tibbetts, S. M., Colombo, S. M., Zanuzzo, F. S., Loveless, A. M., Parrish, C. C., Rise, M. L., Gamperl, A. K., 2022. Influence of supplemental dietary cholesterol on growth performance, indices of stress, fillet pigmentation, and upper thermal tolerance of female triploid Atlantic salmon (*Salmo salar*). Aquacult. Nutr. 2022, 1-18. https://doi.org/10.1155/2022/6336060

IPCC, 2018: Summary for Policymakers. In: *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (Eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 3-24, https://doi.org/10.1017/9781009157940.001.

IPCC, 2022: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., https://doi.org/10.1017/9781009325844.

Johnston, I. A., 1982. Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. Cell. Tissue. Res. 222(2), 325-337. https://doi.org/10.1007/BF00213216

Jones, D. R., 1982. Anerobic exercise in teleost fish. Can. J. Zool. 60(5), 1131-1134. https://doi.org/10.1139/z82-157

Klaiman, J. M., Pyle, G. W., Gillis, T. E., 2014. Cold acclimation increases cardiac myofilament function and ventricular pressure generation in trout. J. Exp. Biol. 217, 4132-4140. https://doi.org/10.1242/jeb.109041

Lin, E., Craig, C., Lamothe, M., Sarunic, M. V., Beg, M. F., Tibbits, G. F., 2015. Construction and use of a zebrafish heart voltage and calcium optical mapping system, with integrated electrocardiogram and programmable electrical stimulation. Am. J. Physiol-Reg I. 308(9), R755-R768. https://doi.org/10.1152/ajpregu.00001.2015

Lurman, G. J., Petersen, L. H., Gamperl, A. K., 2012. In situ cardiac performance of Atlantic cod (*Gadus morhua*) at cold temperatures: Long-term acclimation, acute thermal challenge and the role of adrenaline. J. Exp. Biol. 215(22), 4006–4014. https://doi.org/10.1242/jeb.069849

Minerick, A. R., Chang, H-C., Hoagland, T. M., Olson, K. R., 2003. Dynamic synchronization analysis of venous pressure-driven cardiac output in rainbow trout. A. J. Physiol-Reg. I. 285(4), R889-R896. https://doi.org/10.1152/ajpregu.00228.2003

Morgan, R., Andreassen, A. H., Åsheim, E. R., Jutfelt, F., 2022. Reduce physiological plasticity in a fish adapted to stable temperatures. Proc. Natl. Acad. Sci. 119(22), e2201919119. https://doi.org/10.1073/pnas.2201919119

Nowell, L. B., Brownscombe, J. W., Gutowsky, L. F. G., Murchie, K. J., Suski, C. D., Danylchuk, A. J., Shultz, A., Cooke, S. J., 2015. Swimming energetics and thermal ecology of adult bonefish (Albula vulpes): a combined laboratory and field study in Eleuthera, The Bahamas. Exp. Biol. Fish. 98(11), 2133-2146. https://doi.org/10.1007/s10641-015-0420-6

Olson, K. R., Conklin, D. J., Farrell, A. P., Keen, J. E., Takei, Y., Weaver, L., Smith, M. P., Zhang, Y., 1997. Effects of natriuretic peptides and nitroprusside on venous function in trout. A. J. Phyiol-Reg. I. 273(2), R527-R539. https://doi.org/10.1152/ajpregu.1997.273.2.R527

Reid, C. H., Patrick, P. H., Rytwinski, T., Taylor, J. J., Willmore, W. G., Reesor, B., Cooke, S. J., 2022. An updated review of cold shock and cold stress in fish. J. Fish. Biol. 100(5), 1102-1137. https://doi.org/10.1111/jfb.15037

Rome, L. C., Loughna, P. T., Goldspink, G., 1984. Muscle fiber activity in carp as a function of swimming speed and muscle temperature. Am. J. Physiol-Reg. I. 247(2), R272-R279. https://doi.org/10.1152/ajpregu.1984.247.2.R272

Rome, L. C., Sosnicki, A., Choi, I. H., 1992. The influence of temperature on muscle function in the fast swimming sculp. II. The mechanics of red muscle. J. Exp. Biol. 163(1), 281-295. https://doi.org/10.1242/jeb.163.1.281

Sisson, J. E., Sidell, B. D., 1987. Effect of Thermal Acclimation on Muscle Fiber Recruitment of Swimming Striped Bass (*Morone saxatilis*). Physiol. Zool. 60(3), 310-320. https://doi.org/10.1086/physzool.60.3.30162284

Stoyek, M. R., Quinn, A. T., Croll, R. P., Smith, F. M., 2016. Zebrafish heart as a model to study the integrative autonomic control of pacemaker function. Am. J. Physiol-Heart. C. 311(3), H676-H688. https://doi.org/10.1152/ajpheart.00330.2016

Sutcliffe, R. L., Li, S., Gilbert, M. J. H., Schulte, P. M., Miller, K. M., Farrell, A. P., 2020. A rapid intrinsic heart rate resetting response with thermal acclimation in rainbow trout, *Oncorhynchus mykiss*. J. Exp. Biol. 223, jeb.215210. https://doi.org/10.1242/jeb.215210

Syme, D. A., Gollock, M., Freeman, M. J., Gamperl, A. K., 2008. Power isn't everything: Muscle function and energetic costs during steady swimming in Atlantic Cod (*Gadus morhua*). Phsyiol. Biochem. Zool. 81(3), 320-335. https://doi.org/10.1086/528784

Szekeres, P., Eliason, E., Lapointe, D., Donaldson, M., Brownscombe, J., Cooke, S., 2016. On the neglected cold side of climate change and what it means to fish. Clim. Res. 69(3), 239–245. https://doi.org/10.3354/cr01404

Taylor, E. S., Egginton, S., Taylor, E. W., 1996. Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. J. Exp. Biol. 199(4), 835-845. https://doi.org/10.1242/jeb.199.4.835

Tromp, J. J., Jones, P. L., Brown, M. S., Donald, J. A., Biro, P. A., Afonso, L. O. B., 2018. Chronic exposure to increased water temperature reveals few impacts on stress physiology and growth responses in juvenile Atlantic salmon. Aquaculture. 495, 196–204. https://doi.org/10.1016/j.aquaculture.2018.05.042

Vadboncoeur, E., Nelson, C., Hall, J.R., Clow, K.A., Sandrelli, R.M., Brauner, C.J., Swanson, A.K., Gamperl, A.K., (in final prep). Lowering temperature to 1˚C results in physiological changes, stress and mortality in cultured Atlantic salmon (*Salmo salar*). Aquaculture.

Vornanen, M., 1998. L-type Ca^{2+} current in fish cardiac myocytes: effects of thermal acclimation and beta-adrenergic stimulation. J. Exp. Biol. 201(4), 533-547. https://doi.org/ 10.1242/jeb.201.4.533

Vornanen, M., 2016. The temperature dependence of electrical excitability in fish hearts. J. Exp. Biol. 219(13), 1941-1952. https://doi.org/10.1242/jeb.128439

Vornanen, M., 2017. Electrical Excitability of the Fish Heart and its Autonomic Regulation. In: Gamperl, A. K., Gillis, T. E., Farrell, A. P., Brauner, C. J., (Eds.). Fish Physiology, vol 36, pp. 99-153. Elsevier. https://doi.org/10.1016/bs.fp.2017.04.002

Vornanen, M., Ryökkynen, A., Nurmi, A., 2002. Temperature-dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. Am. J. Phys. Reg. I. 282(4), R1191-R1199. https://doi.org/10.1152/ajpregu.00349.2001

Zhang, Y., Healy, T. M., Vandersteen, W., Schulte, P. M., Farrell, A. P., 2018. A rainbow trout (*Oncorhynchus mykiss*) strain with higher aerobic scope in normoxia also has superior tolerance of hypoxia. J. Fish. Biol. 92(2),

APPENDIX

Table S1. Morphometric data (mean ± 1 s.e.m. with n = 9 per group) for all fish used during the experiment performed in Chapter 2. K = condition factor; RVM = relative ventricular mass.

	8°C Acclimated	**1°C Acclimated**	**8-1°C**	**p-value**
Weight (kg)	1.3±1.2	1.03±0.8	1.1±0.7	0.210
Length (cm)	44.1±1.2	41.1±0.8	41.7±0.8	0.962
K	1.45±0.080	1.46±0.030	1.50±0.050	0.869
RVM (%)	0.0805±0.00586	0.0699±0.00252	0.0732±0.00884	0.500

Table S2. Results for the general linear mixed effects model used to test the main effects of acclimation group and time during the experiment in Chapter 2. These measurements were on resting salmon.

Cardiorespiratory Variable	**Main Effect**	**df**	**F**	***P***
f_H	Acclim	2	142.850	<0.0001
	Time	7	84.580	<0.0001
	Acclim x Time	14	118.540	<0.0001
$\dot{Q}$	Acclim	2	13.675	0.0003
	Time	7	3.445	0.002
	Acclim x Time	14	2.425	0.005
V_S	Acclim	2	0.238	0.791
	Time	7	0.307	0.950
	Acclim x Time	14	0.926	0.533
$\dot{M}O_2$	Acclim	2	12.455	0.0002
	Time	7	5.898	<0.0001
	Acclim x Time	14	3.556	<0.0001
$\dot{M}O_2 / \dot{Q}$	Acclim	2	0.624	0.548
	Time	7	0.854	0.545
	Acclim x Time	14	1.166	0.310

Provided are the degrees of freedom (df), the F-statistic, and *P*-values for main the effects of Acclim = acclimation group, Time = point in the experiment when measurements were made, and Acclim x Time = the interaction between these main effects. The parameters are f_H = heart rate, $\dot{Q}$ = cardiac output, V_S = stroke volume, $\dot{M}O_2$ = oxygen consumption, and $\dot{M}O_2 / \dot{Q}$ = O_2 extraction.

Table S3. Results of the general linear mixed effects model and one-way ANOVA (relative V_S) used to test the main effects of acclimation group and pharmacological antagonists on cardiac variables in resting Atlantic salmon in Chapter 2.

Cardiac Variable	Main Effect	df	F	*P*
f_H	Acclim	2	319.966	< 0.0001
	Pharma	2	64.816	< 0.0001
	Acclim x Pharma	4	1.627	0.178
$\dot{Q}$	Acclim	2	18.380	< 0.0001
	Pharma	2	1.825	0.180
	Acclim x Pharma	4	1.687	0.181
V_S (ml kg^{-1})	Acclim	2	3.586	0.0489
	Pharma	2	1.093	0.348
	Acclim x Pharma	4	3.430	0.0201
V_S (volts $beat^{-1}$)	Acclim	2	5.999	< 0.01
	Pharma	2	3.971	0.0265
	Acclim x Pharma	4	5.888	< 0.001
Relative V_S After Atropine	8°C Acclimated		5.558	0.0116
	1°C Acclimated			
	8-1°C			
Relative V_S After Propranolol	8°C Acclimated		9.815	< 0.01
	1°C Acclimated			
	8-1°C			

Provided are the degrees of freedom (df), the F-statistic, and the *P*-values for main effects of Acclim = acclimation group, Pharma = pharmacological antagonist, and Acclim x Pharma = the interaction between these main effects. Parameters are f_H = heart rate, $\dot{Q}$ = cardiac output, V_S = stroke volume (mL kg^{-1} and volts $beat^{-1}$), and *Relative* V_S = relative stroke volume (%). The data for the last 3 parameters are shown in Figure S1.

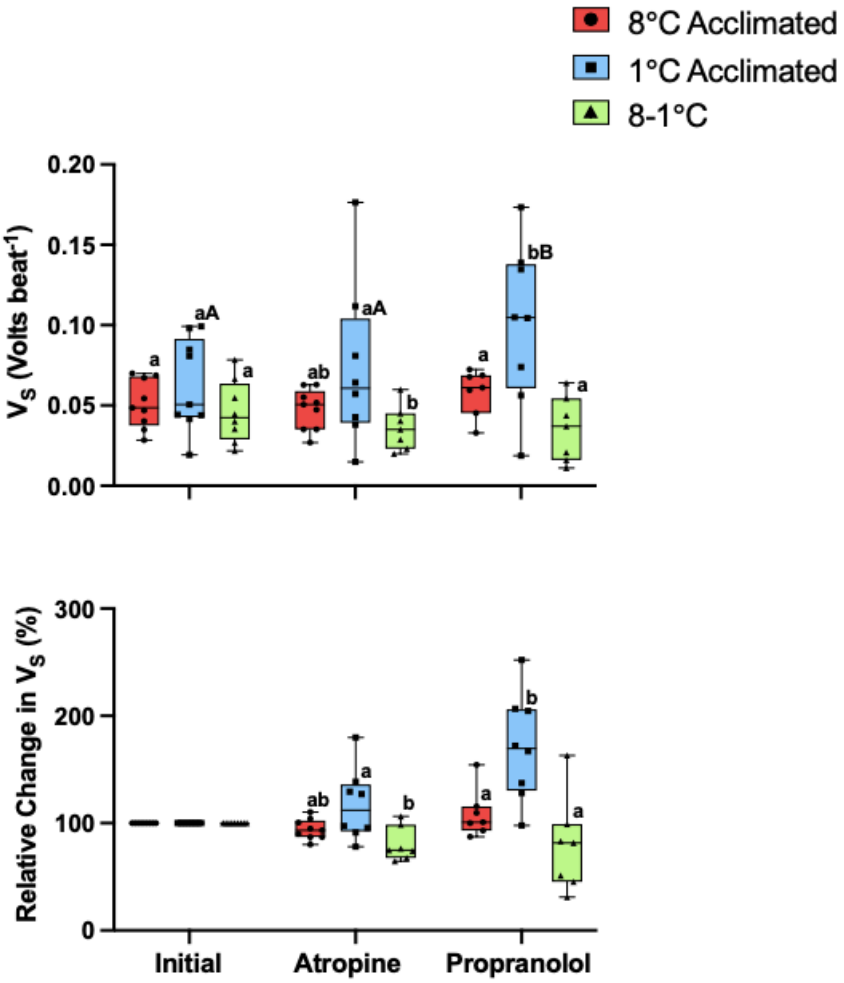

Figure S1. Stroke volume (V_S in volts beat^{-1}) and relative changes in stroke volume (V_S in %) in Atlantic salmon acclimated to 8 (*red*) and 1°C (*blue*), and acutely cooled from 8 to 1°C (*green*), when injected with the pharmacological antagonists atropine sulfate (to block cholinergic nervous tone) and propranolol hydrochloride (to block $ß_1$- and $ß_2$-adrenoreceptors) in Chapter 2. Symbols without a letter in common are significantly different ($p < 0.05$) between groups (lowercase) and between the pharmacological antagonists within a group (uppercase). Values are means ± 1 s.e.m. with n = 7-9 per group. See Table S3 for statistical information.

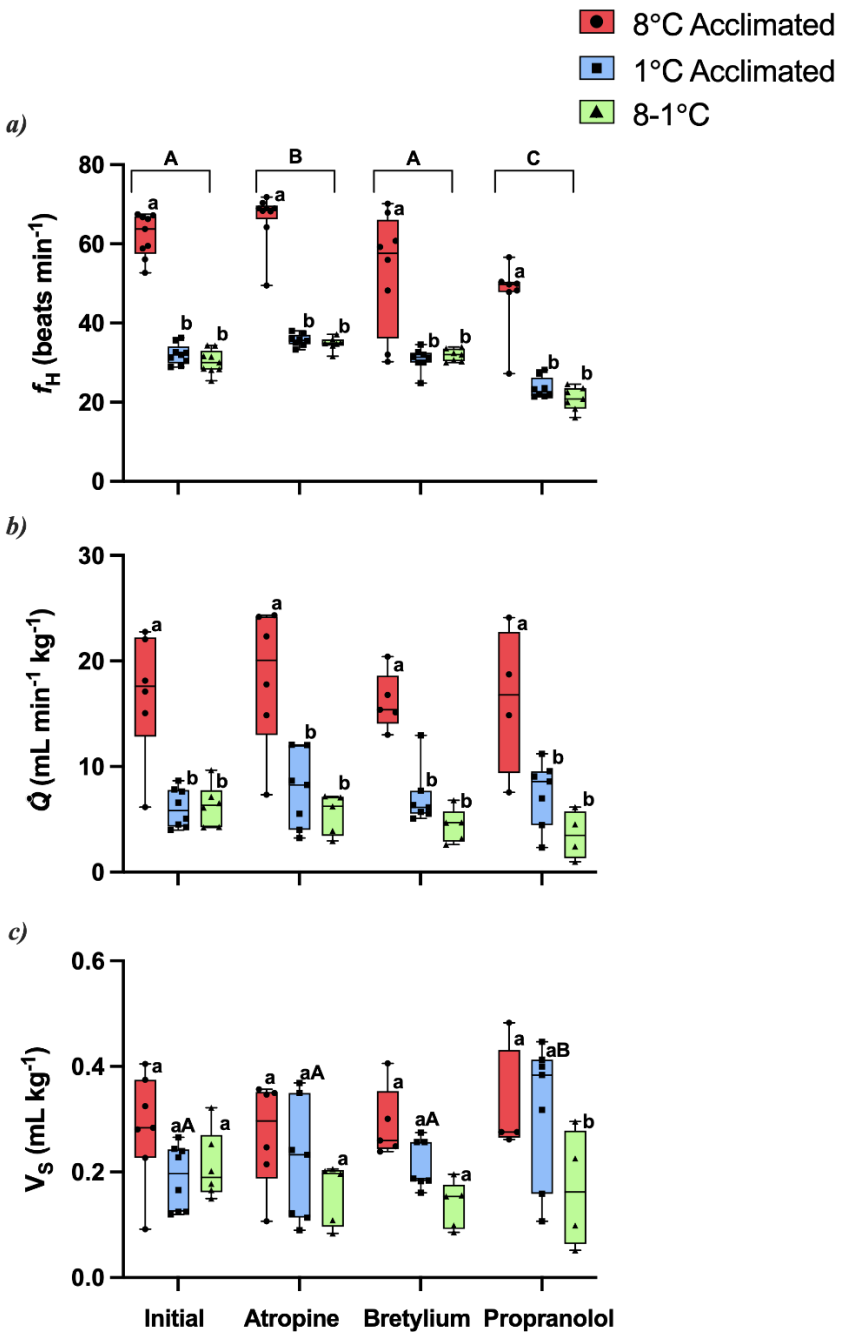

Figure S2. Changes in cardiac function [(*a*) heart rate (f_H), (*b*) cardiac output ($\dot{Q}$), and (c) stroke volume (V_S)] in Atlantic salmon acclimated to 8 (*red*) and 1°C (*blue*), and acutely cooled from 8 to 1°C (*green*), when injected with the pharmacological antagonists atropine sulfate, bretylium tosylate, and propranolol hydrochloride in Chapter 2. Only 45 minutes were allowed between the injection of the pharmacological antagonists, and thus, the effects of bretylium may not have been fully realized (see Methods section). Symbols without a letter in common are significantly different ($p < 0.05$) between groups (lowercase) and between the pharmacological antagonists within a group (uppercase). Values are means ± 1 s.e.m. with n = 5-9 per group.

Table S4. Morphometric data (mean ± s.e.m.) for all fish used in Chapter 3 based on acclimation temperature and sex as fixed factors. Values of RVM (relative ventricular mass) without a letter in common are significantly different between acclimation groups ($p < 0.05$). No significant differences between male and female fish were found. Values are means ± 1 s.e.m.

	8°C	**4°C**	**1°C**
Weight (g)			
Male	717.1 ± 22.4	745.8 ± 99.0	665.6 ± 37.7
Female	653.7 ± 21.01	689.0 ± 41.7	559.7 ± 53.6
Length (cm)			
Male	38.1 ± 0.5	38.3 ± 1.5	37.7 ± 0.8
Female	37.3 ± 0.5	37.6 ± 0.6	35.0 ± 1.3
K			
Male	1.29 ± 0.042	1.30 ± 0.036	1.24 ± 0.039
Female	1.26 ± 0.032	1.31 ± 0.026	1.21 ± 0.113
RVM (%)			
Male	0.0857 ± 0.0030[a]	0.0926 ± 0.0056 [b]	0.0968 ± 0.0058 [b]
Female	0.0812 ± 0.0030 [a]	0.102 ± 0.0075 [b]	0.103 ± 0.0038 [b]

Table S5. Results for the two-way ANOVAs used to test for significant differences in the morphometric data (mean ± s.e.m.) for all fish used in Chapter 3. Fixed factors were acclimation temperature and sex.

Morphometric	**Main Effect**	**Df**	**F**	***P***
Weight	Acclim	2	1.962	0.159
	Sex	1	3.984	0.0557
	Acclim x Sex	2	NA	NA (0.839)
Length	Acclim	2	1.192	0.319
	Sex	1	4.012	0.0549
	Acclim x Sex	2	NA	NA (0.419)
K	Acclim	2	1.112	0.343
	Sex	1	0.285	0.598
	Acclim x Sex	2	NA	NA (0.925)
RVM	Acclim	2	8.015	0.00177
	Sex	1	0.148	0.703
	Acclim x Sex	2	NA	NA (0.276)

**If the interaction term was not significant (NA) it was taken out of the model and the model was re-run.*

Table S6. The values for resting, maximum, absolute scope and factorial scope for heart rate (f_H; beats min^{-1}), cardiac output ($\dot{Q}$; mL min^{-1} kg^{-1}), stroke volume (V_S; mL kg^{-1}) and tissue-oxygen extraction ($\dot{M}O_2 / \dot{Q}$; mg O_2 mL $blood^{-1}$) and the critical swim speed speed (U_{crit}; bL s^{-1}) for salmon acclimated to 8, 4 and 1°C and acutely cooled from 8 to 1°C in Chapter 3. Values are means ± 1 s.e.m. and those without a letter in common are significant at $p < 0.05$; n = 7-9 per group.

		8°C	4°C	1°C	8 to 1°C
f_H					
	Rest	64.52± 1.40 [a]	45.49 ± 2.24 [b]	34.14 ± 1.23 [c]	27.16 ± 1.03 [d]
	Max	77.90 ± 0.87 [a]	57.97 ± 0.69 [b]	40.57 ± 0.72 [c]	37.88 ± 0.89 [c]
	AbS	13.37 ± 1.47 [a]	12.48 ± 2.06 [a]	6.42 ± 0.71 [b]	10.72 ± 0.93 [ab]
	FS	1.21 ± 0.03 [a]	1.29 ± 0.06 [ab]	1.19 ± 0.03 [a]	1.40 ± 0.05 [b]
$\dot{Q}$					
	Rest	21.37 ± 2.11 [a]	17.61 ± 1.39 [a]	9.07 ± 0.45 [b]	18.62 ± 1.71 [a]
	Max	39.47 ± 3.09 [a]	40.61 ± 2.74 [a]	27.79 ± 0.79 [b]	28.73 ± 2.50 [b]
	AbS	19.48 ± 1.78 [a]	23.00 ± 3.03 [a]	18.72 ± 0.75 [a]	10.12 ± 0.87 [b]
	FS	1.98 ± 0.14 [ac]	2.42 ± 0.30 [ab]	3.11 ± 0.16 [b]	1.55 ± 0.02 [c]
V_S					
	Rest	0.333 ± 0.038 [a]	0.394 ± 0.036 [a]	0.269 ± 0.019 [a]	0.669 ± 0.081 [b]
	Max	0.546 ± 0.043 [a]	0.734 ± 0.041 [ab]	0.701 ± 0.018 [ab]	0.811 ± 0.074 [b]
	AbS	0.230 ± 0.017 [ac]	0.370 ± 0.060 [a]	0.424 ± 0.017 [b]	0.158 ± 0.037 [c]
	FS	1.75 ± 0.10 [a]	2.02 ± 0.31 [ac]	2.68 ± 0.17 [b]	1.26 ± 0.08 [c]
$\dot{M}O_2 / \dot{Q}$					
	Rest	0.050 ± 0.0062 [a]	0.048 ± 0.0047 [a]	0.088 ± 0.0058 [b]	0.041 ± 0.0050 [a]
	Max	0.168 ± 0.012	0.128 ± 0.014	0.135 ± 0.009	0.117 ± 0.017
	AbS	0.112 ± 0.009 [a]	0.081 ± 0.013 [ab]	0.047 ± 0.011 [b]	0.076 ± 0.014 [ab]
	FS	3.42 ± 0.26 [a]	2.86 ± 0.33 [a]	1.58 ± 0.16 [b]	2.90 ± 0.33 [a]

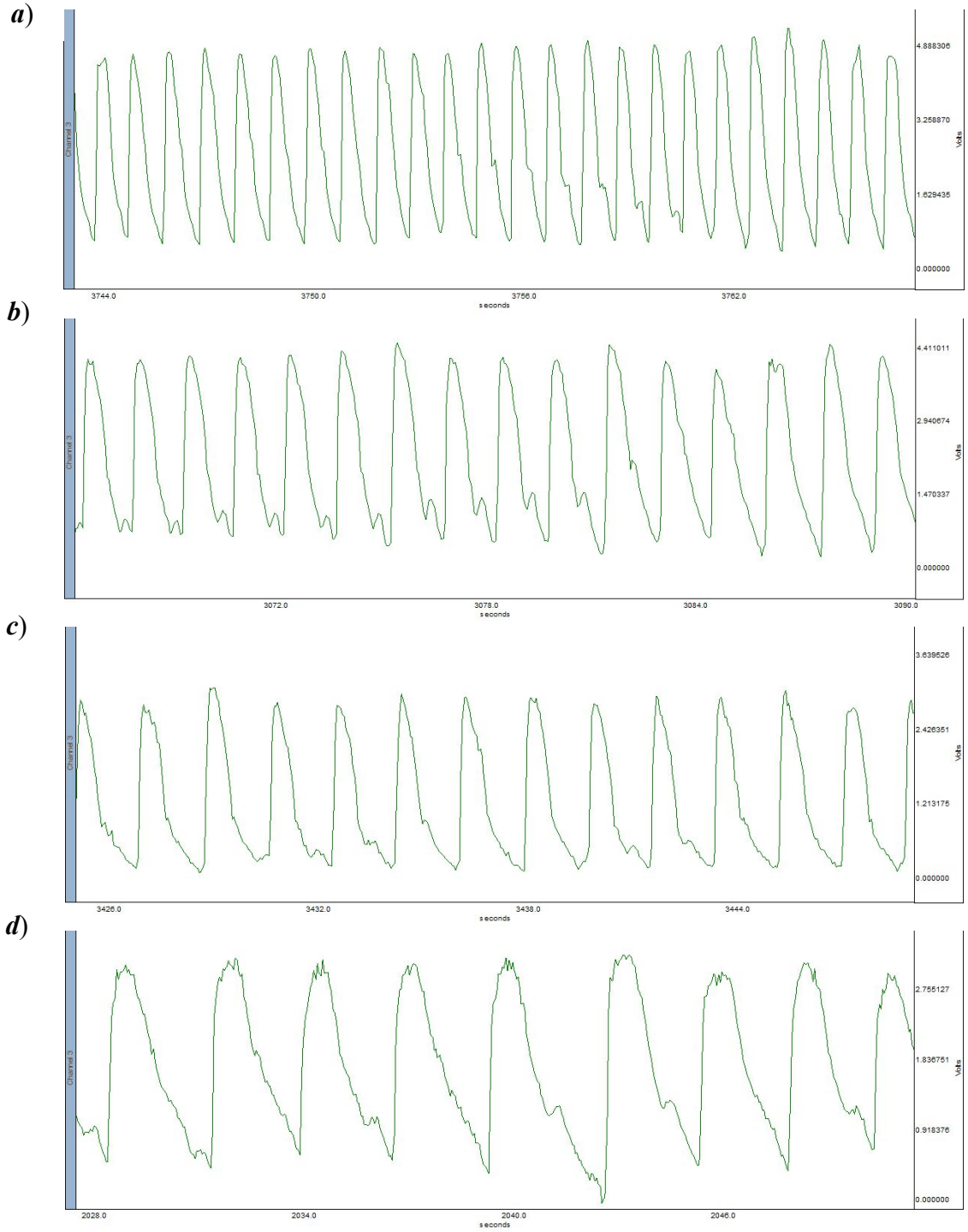

Figure S3. The resting heart rate (f_H; beats min^{-1}) of Atlantic salmon: (*a*) acclimated and tested at 8°C, (*b*) acclimated and tested at 4°C, (*c*) acclimated and tested at 1°C and (*d*) acclimated to 8 and tested at 1°C. All tracings were recorded prior to the critical swim speed (U_{crit}) test in Chapter 3.