THE EFFECT OF ACCLIMATION TEMPERATURE AND TRIPLOIDY ON HYPOXIA TOLERANCE IN BROOK CHARR, *SALVELINUS FONTINALIS*

by

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ABSTRACT

Triploid fish could be beneficial to aquaculture sustainability due to their effective sterility preventing escaped farmed fish from mating with wild fish. However, experience to date has suggested that they are less tolerant of environmental stressors. The goal of this study was therefore to determine whether acclimation to warm temperature improves the performance of both diploid and triploid brook charr (*Salvelinus fontinalis*) under conditions of high temperature and hypoxia. A preliminary experiment tested fish of both ploidies acclimated to two different temperatures (15 and 18°C) at a range of test temperatures (ambient, 20, 22, 24, 26, 28, 30°C) to determine the oxygen tension (PO_2) at loss of equilibrium and time taken to reach loss of equilibrium, during progressive hypoxia. A follow-up experiment involved first acclimating fish to the same two temperatures and then reacclimating the 18°C fish to 15°C before using the same protocol to test hypoxia tolerance at a narrower range of temperatures (ambient, 24, 26, 28, 30°C). Warm acclimation (18°C) improved high temperature and hypoxia tolerance in both ploidies, but this improvement did not last after reacclimation to cooler temperatures. Triploids had slightly lower hypoxia tolerance in both experiments. This study shows that (1) while increasing acclimation temperature improves tolerance of fish regardless of ploidy in high temperature and hypoxic conditions, the effect is not long-lasting, and (2) the difference in tolerance between ploidies may not be great enough for triploids to have a negative impact on the aquaculture industry and instead should be used to minimize negative impacts caused by farmed salmon mating with wild populations of Atlantic salmon. However, further research needs to be done to optimize this approach for use in the aquaculture industry.

DEDICATION

This thesis is dedicated to Billy Jensen, for all you do.

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1. Introduction

1.1: Aquaculture in Atlantic Canada

Salmonid species are of cultural and economic importance in Atlantic Canada. Atlantic salmon (*Salmo salar*) have been fished for sport as well as commercially for centuries (Dunfield, 1985). The commercial fishing industry was highly successful until overfishing, habitat destruction, and blocking riverways began to take its toll on the populations in our region (Lucas, 2012). This led to the decline of the commercial fishery which was shut down in New Brunswick in 1985 (Cook and McGaw, 1991). However, the demand for salmon was still present and therefore the best option to meet this was the introduction and implementation of floating net-pen aquaculture (Purser and Forteath, 2012). This method relies on the cultivation of eggs into parr from broodstock in land-based freshwater hatcheries until the parr undergo smoltification (Purser and Forteath, 2012). After this, they are moved to the ocean into the floating net-pens for approximately 18 months, after which they have reached market size and can be harvested. The first harvest of Atlantic salmon in the Bay of Fundy occurred in 1978 (Sutterlin *et al.*, 1981). Since then the industry has grown to a production value of $270 million in 2018 (Government of New Brunswick, 2019).

This solution to meeting the demand for salmon has not been without issue because net-pens are prone to developing rips and tears (Jensen *et al.*, 2010). As time has passed, technology has improved, but nothing is infallible and the tearing of the net-pens allows farmed fish to escape (Jensen *et al.*, 2010). These escapees are problematic

because of the ongoing struggle of wild Atlantic salmon populations to survive, despite the efforts of conservation groups (Committee on the Status of Endangered Wildlife in Canada, 2010). Escaped farmed fish have been shown to travel up rivers and spawn with wild Atlantic salmon (McGinnity *et al.*, 1997; 2003, Wringe *et al.*, 2018). This leads to a modification of the wild Atlantic salmon genome, referred to as genetic introgression (Glover *et al.*, 2013; Heino *et al.*, 2015; Glover *et al.*, 2018). This concept outlines how farmed fish are selectively bred to express certain financially beneficial phenotypes such as body size, fillet quality, and age at sexual maturation (Glover *et al.*, 2013; 2018). While beneficial to the fish farmer, these traits do not benefit wild populations of Atlantic salmon, and the addition of these selected alleles could further harm wild salmon populations (Glover *et al.*, 2013; 2018). Atlantic salmon farm escapees have been found upwards of 100km from their escape site (Wringe *et al.*, 2018).

1.2: Triploidy as a Solution

While aquaculture technology continues to improve to reduce the likelihood of farmed fish escaping, the fact is that with millions of farmed fish in a relatively unsupervised environment, it is inevitable that escapes will occur (Jensen *et al.*, 2013). Many solutions to the problem of farmed fish mating with wild fish have been proposed such as attempting to trap, angle for, or poison escapees, but these will never completely eradicate farmed fish from the environment. These methods also cannot be even moderately successful without an impact on wild populations, as wild fish would be susceptible to capture. It would also be impossible to ensure the fish were captured within a short time from their escape. If they were caught after they had already mated with wild

fish, then they will have already impacted the wild populations (Skilbrei and Jørgensen, 2010; Chittenden *et al.,* 2011). A good measure to prevent this would be the use of reproductively sterile stocks of fish for aquaculture that can have little genetic impact on wild fish.

One of the proposed methods to create reproductively sterile fish is the use of triploid populations for aquaculture. Triploids are organisms with three full sets of chromosomes, making them effectively sterile. Triploidy occurs naturally in some plant and amphibian species, as well as a few species of fish such as Prussian carp, *Carassius gibelio,* and weather loach, *Misgurnus anguillicaudatus* (Piferrer *et al.*, 2009).

Anesthetics and temperature treatments have been used successfully to produce triploids, but the method that is the most viable for commercial production while also being cost-effective is the use of hydrostatic pressure (Piferrer *et al.,* 2009). These treatments are applied to fertilized eggs when the second polar body would normally be extruded (Piferrer *et al.*, 2009). The second polar body contains a set of maternal chromosomes, and its retention results in the organism having three full sets of chromosomes (Piferrer *et al.,* 2009). Triploidy induction via hydrostatic pressure requires the purchase of readily available equipment, and the determination of the correct timing and duration of the pressure shock. For example, for Atlantic salmon it is 5 min at 65.5 MPa beginning 300°C-min after fertilization (Benfey and Sutterlin, 1984). This has been highly successful in the production of triploids on a commercial scale (Benfey, 2016).

Triploid fish are still capable of producing gametes, but these are generally aneuploid (i.e., the number of chromosomes is not a multiple of haploid) and therefore

any embryos derived from them will die early in development (Li *et al.*, 2016). This is beneficial to the aquaculture industry because if triploid fish escape, any attempt to breed with wild fish will not produce viable offspring. Ideally all-female triploid populations are used because unlike males, triploid females do not exhibit mating behaviour and therefore would have no effect on successful spawning of wild Atlantic salmon (Benfey, 2016).

Despite the potential benefits to using triploids, the aquaculture industry has not adopted them on a broad scale and triploid Atlantic salmon are only widely used in Tasmania. A single North American company also uses them only due to the salmon they farm being transgenic and therefore assessed as being a greater genetic risk to wild salmon (Thorstad *et al.*, 2008; Waltz, 2017). Most countries, including the world's largest aquaculture producers, do not use them at all. The reasons behind the decision not to use triploids include things such as the tendency of triploids to underperform in conditions of high temperature or hypoxia (Ojolick *et al.*, 1995; Altimiras *et al*., 2002; Hansen *et al.*, 2015). However, some studies have shown no difference in their performance compared to diploids, and other studies have shown triploids to outperform diploids (Ellis *et al.*, 2013; Scott *et al.*, 2015; Benfey and Devlin, 2018, Bowden *et al.*, 2018). These inconsistencies among studies could be due to a multitude of factors, such as different techniques for testing fish, as well as different pre-trial husbandry techniques.

Other reasons that triploids are not widely used for aquaculture is that they have a higher occurrence of jaw, gill and spinal deformities and of cataracts (Wall and Richards, 1992; Madsen *et al.*, 2000; Sadler *et al.*, 2001; Fjelldal and Hansen, 2010). A similar

problem existed for diploid fish when they were first farmed and improvements to husbandry techniques have mitigated these problems. For instance, studies of triploid nutritional requirements led to a decrease in these deformities through dietary supplementations of phosphorous and histidine in triploid-specific diets (Taylor *et al.*, 2015; Peruzzi *et al.,* 2018). This requirement may be due to triploids having a slightly different gut configuration when compared to diploids, with triploids having a shorter gut and different number of pyloric caeca (Peruzzi *et al.,* 2014). Further studies have also shown triploids to have a lesser number of vertebrae than diploids but increased dietary phosphorous decreased the incidence of vertebral deformity (Peruzzi *et al.,* 2018). Triploids have also shown decreased cataract occurrence when fed diets containing higher levels of histidine (Taylor *et al.*, 2015).

This all leads to a great need for improved triploid husbandry techniques. While triploids are not necessarily new, they have largely been raised the same as diploids in study settings as well as small-scale aquaculture studies (e.g., McGeachy *et al.* 1995; O'Flynn *et al.,* 1997). The reluctancy to adopt triploids for large-scale use seems to be influenced by the stigma surrounding their poor performance, as well as a negative consumer perception that they are genetically modified (Ferguson *et al.*, 2007). Diploid husbandry has been steadily improved for decades, leading to their great success in salmonid aquaculture (e.g., Jobling *et al.*, 2010). If the same were done for triploids, they may have a better survival rate and performance in aquaculture, meaning they would no longer be detrimental to the companies electing to use them. This means that a determination of optimal triploid husbandry is important in all aspects, from diet to rearing temperature.

1.3: Hypoxia Tolerance

Hypoxia tolerance has been widely studied in many species of fish. The importance of hypoxia tolerance is usually related to the relationship between hypoxia and temperature: as the temperature of water increases, the solubility of gasses such as oxygen decreases (Dejours, 1975). This means that the warmer the water is, the less oxygen is available. With warming sea surface temperatures, this could have a significant impact on the ability for fish to be farmed in the ocean. Recent studies of freshwater temperature increase also indicates a warming trend, which could likely be correlated with an increase in hypoxia events, and impacting both farmed and wild fish (Intergovernmental Panel on Climate Change, 2013).

By tagging fish in net-pens with oxygen sensors that track the amount of oxygen available to the fish as well as their depth within the net-pen, several studies have shown that farmed fish are frequently exposed to hypoxic conditions (Dempster *et al.,* 2016; Stehfest *et al.,* 2017; Solstorm *et al.,* 2018). Stehfest *et al.* (2017) found that within the net-pens, fish were avoiding the hypoxic bottom and warmer surface water of the cage, meaning that the fish were not utilizing the full space available to them. This highlights an important challenge to farmed fish due to the fixed nature of net-pens because the natural response of a cold-water fish such as salmon to a high temperature or hypoxia event is to seek cold-water refugia (Breau *et al.,* 2007; 2011). This response is also shown within the net-pens, with the fish selecting a "habitat" within the net-pen based on temperature and oxygen saturation, which can be as low as 21% (Dempster *et al.,* 2016). This "habitat selection" results in the fish crowding within a narrow depth range (4-6m)

to avoid non-ideal conditions. In light of such scenarios, for the industry to use triploid fish for aquaculture, they must perform similarly if not better than diploids in hypoxic conditions to prevent financial loss associated with their use.

For this study, brook charr (*Salvelinus fontinalis)* were used. Brook charr are a cold-water salmonid, which means their thermal tolerance range is relatively small. Their critical thermal maximum is about 29°C at high oxygen saturations for both diploids and triploids but is influenced by rearing temperature and the age of the fish (Benfey *et al.*, 1997; Stitt *et al.*, 2013). Previous work has shown that as temperature increases, hypoxia tolerance also decreases for both diploids and triploids (Benfey and Devlin, 2018). The dependence of high temperature tolerance on oxygen availability is commonly referred to as oxygen and capacity limited thermal tolerance (OCLTT) (Pörtner, 2001; 2002; Pörtner *et al*., 2017). This concept outlines how basic molecular processes can determine whole-animal responses to increased temperature in terms of their aerobic scope, and how these are dependent on oxygen availability (Pörtner *et al*., 2017). The validity of this concept has been widely debated, and other studies have shown that increasing acclimation temperature can improve aerobic performance regardless of oxygen availability (Gräns *et al*., 2014).

Previous work has also shown that there is an inflection point where increasing temperature causes hypoxia tolerance to drop dramatically (e.g., Benfey and Devlin, 2018) and it is important to examine a range of temperatures to determine where this point lies. This relationship may differ between ploidies (Benfey and Devlin, 2018), and may also be influenced by acclimation temperature. An effective way to test a fish's

tolerance to high temperature and hypoxia is through the non-lethal loss-of-equilibrium response (e.g., McKenzie *et al.*, 2003; Mandic *et al.*, 2009; Scott *et al.*, 2015; Benfey and Devlin, 2018; Bowden *et al.*, 2018).

Loss of equilibrium has been used to study hypoxia tolerance in many fish species including salmonids (Wendelaar Bonga, 1997). The physiological pathway that leads to this response remains unclear, however, it is influenced by hypoxia and provides a good indication of when the physiological stress is too much. To appropriately measure this response for this research, the oxygen tension (PO_2) at loss of equilibrium was used as a proxy for hypoxia tolerance rather than dissolved oxygen (DO) concentration or percent air saturation because oxygenation of the blood is dependent on a pressure-based diffusion gradient (Evans *et al.*, 2005), making PO_2 a more accurate measurement of a fish's ability to tolerate hypoxia (Ultsch and Nordlie, 2019).

Hypoxia tolerance has been correlated with many different physiological parameters. One of these is the blood's concentration of hemoglobin (Lai *et al.*, 2006), the protein found in red blood cells that binds oxygen molecules for transport to metabolically active cells. The amount of hemoglobin found in the blood is an indication of how much oxygen the organism is able to transport within the body and therefore predicts the overall carrying capacity of oxygen in the blood (Mandic *et al.*, 2009). Other blood parameters, such as hematocrit and red blood cell count, offer further insight into oxygen carrying capacity (Mandic *et al.*, 2009) and can be used to calculate the mean red blood cell volume and hemoglobin content and concentration. Hypoxia exposure has been shown to increase hemoglobin concentration and thus the oxygen carrying capacity of the blood (Mandic *et al.*, 2009).

1.4: Temperature Acclimation

Temperature plays an important role in driving many physiological processes within living organisms, especially ectotherms like fish. The thermal window within which fish can live is usually determined by the scope at which internal biochemical reactions can be adequately catalyzed, otherwise known as their metabolism (Pörtner, 2010). This range can shift depending on environmental changes, for example, as with the predicted mean global temperature increase of 2-4°C (Intergovernmental Panel on Climate Change, 2013). This may result in the redistribution of many fish species, as their thermal tolerance windows will not allow them to thrive within their former geographical ranges (Wehrly *et al.*, 2003).

Different physiological responses occur when fish are exposed to acute thermal stress, including increased plasma cortisol and lactate levels. These trigger a cascade of responses such as increased heart and respiration rates, until the thermal stress reaches a point where the fish can no longer take it and they lose equilibrium, followed shortly after by death (Wendelaar Bonga, 1997). This point is known as the upper lethal temperature.

The standard metabolic rate (SMR) of ectotherms increases exponentially with temperature until the upper lethal temperature is reached (Pörtner, 2010). This is a result of the increase in energy requirements to support basic maintenance functions brought on by the change in temperature. This increase in SMR leads to a decrease in aerobic scope, which is defined as the difference between SMR and maximum metabolic rate (MMR). With a higher SMR but the MMR declining as temperature approaches upper lethal temperature, the aerobic scope is diminished (Sandblom *et al.*, 2014). However, fish can

show a decrease in SMR over time with prolonged exposure to higher temperatures, suggesting that they can acclimate to increased temperature within a window of thermal tolerance (Sandblom *et al.,* 2014) such that aerobic scope is no longer diminished.

Triploid fish have shown a reduced aerobic scope which leads to a reduced capacity to tolerate increased temperature (Bowden *et al.,* 2018). This reduced aerobic scope is thought to be due to an elevated SMR which, when coupled with a similar MMR, means that the overall aerobic scope is smaller (Lijalad and Powell, 2009). The question remains whether the aerobic performance of triploids at high temperature can be improved through acclimation. Some studies suggest that this may be the case, however, temperature acclimation studies of triploids have given inconsistent results (Atkins and Benfey, 2008; Ellis *et al.,* 2013; Scott *et al.,* 2015). These inconsistencies may arise from different rearing parameters, different age classes of fish, and different salmonid species being utilized for these studies.

Acute thermal stress has a more negative impact on physiology when compared to gradually increasing water temperature, the impact of which is dependent on the species' ability to acclimate to a range of temperatures (Pörtner, 2010). Thermal acclimation to slightly higher temperatures has been shown to improve performance in shorter challenges of very high temperatures in fish species such as landlocked Atlantic salmon (*Salmo salar* m. *Sebago*) (Anttila *et al.,* 2015), Arctic charr (*Salvelinus alpinus*) (Anttila *et al.,* 2015), and brook charr (McCormick *et al.,* 1972) as well as non-salmonid species such as Atlantic sturgeon (*Acipenser oxyrinchus*) (Secor and Gunderson, 1998) and Atlantic killifish (*Fundulus heteroclitus*) (McBryan *et al.,* 2016).

Anttila *et al.* (2014) showed that Atlantic salmon can have their thermal tolerance improved when acclimated to 20°C after the start of exogenous feeding, as demonstrated by a 4.5°C increase in the temperature at which cardiac arrythmias begin. This is a common theme among thermal tolerance studies on salmonids, with juvenile cutthroat trout (*Oncorhychus clarkii pleuriticus*) improving their critical thermal maximum (CT_{max}) by 3°C after acclimation to higher temperatures for approximately 40 days (Underwood *et al.*, 2012). Two studies on adult lake charr (*Salvelinus namaycush*) by McDermid *et al.* (2013) and Kelly *et al*. (2014) found that acclimation to warmer temperature increased the CT_{max} by 3°C. Studies done on brook charr have also shown a statistically significant improvement in the upper thermal tolerance following acclimation to higher temperature (Stitt *et al.*, 2013). MacNutt *et al.* (2004) also found that a temperature acclimation period of 48h versus 3 weeks yielded very similar results in swimming tests on cutthroat trout, indicating that the time fish are acclimated to increased temperatures may not have a significant effect on the improvement in performance beyond a minimum time frame, such as 48h in this example. However, it is important to note that the long-term effects after acclimation are not clear. The improvement in thermal tolerance with acclimation may only be temporary, and the long-term effects should be examined.

1.5: Objective, Hypotheses, and Significance

This thesis tested the hypothesis that ploidy and previous acclimation temperature will affect the fish's response to hypoxia at high temperatures. This was done by examining the effect of acclimation temperature on hypoxia tolerance in diploid and

triploid brook charr at various test temperatures. Any indication of ploidy-specific thermal optima will be useful in developing production protocols for improving the aquaculture performance of triploid salmonids. It was predicted that acclimating triploid fish to higher temperatures will improve their hypoxia tolerance at higher temperatures, bringing it closer to that of diploids.

This study also aimed to examine a variety of physiological parameters to determine whether they predicted individual differences in hypoxia tolerance. The physiological pathway that leads to the difference in hypoxia tolerance between ploidies remains largely unclear. If this pathway is made known, it could lead to improved husbandry techniques not only for triploids, but for other fish species used in aquaculture.

Brook charr were selected as a model salmonid rather than the more aquaculture-relevant Atlantic salmon because of their smaller size, given that the Aquatic Facility at UNB is not suitable for raising post-smolt Atlantic salmon. They are also a commercially and culturally important species in our region in their own right, and triploid brook charr are used in stocking programs by the Government of New Brunswick. This means that determining the hypoxia and temperature tolerance of this species provides useful information that goes beyond the problem with aquaculture and Atlantic salmon. Juveniles were used because their smaller size made it possible to house the large number required for these experiments. Furthermore, using juveniles removes sexual maturation as a confounding factor.

1.5.1 Experiment 1

The specific objective for the first experiment was to establish whether there was an improvement in hypoxia tolerance for fish acclimated to higher temperatures. This study was designed as a proof of concept, i.e., that acclimation does improve hypoxia and high temperature tolerance in salmonid fish as shown in other studies. The study was also designed to examine whether ploidy impacts the effect of temperature acclimation on hypoxia tolerance. Other studies on triploid salmonids give mixed reviews on their performance, therefore this study aimed to provide additional evidence as to whether they underperform, and if their performance is improved with temperature acclimation. Additionally, this study tested fish at a range of temperatures to determine whether any effects present are temperature dependent.

1.5.2 Experiment 2

The specific objective for the second experiment was to determine if the effects of acclimating fish to higher temperatures remain consistent over time once fish are removed from this higher rearing temperature. This study was based on the information from past literature that acclimation to higher temperature would improve hypoxia tolerance. After determining how large the effect of temperature acclimation was from the first experiment, the second experiment aimed to determine if this effect was still present after removing fish from acclimation, or if it declined. If the effect is reduced, this study also aims to determine how much the effect is reduced by. This study also determined if the potential reduction in effects is impacted by ploidy, and other physiological parameters measured in this study.

2. Methods

2.1 Experiment 1

2.1.1 Rearing Information

This research was approved by the UNB Animal Care Committee (Animal Use Protocol 19014), following the guidelines of the Canadian Council on Animal Care. The fish were produced in December 2018 as progeny of several adult brook charr raised in the UNB Aquatic Facility. Triploidy was induced in half of the eggs by pressure treatment at 65.5 MPa for 5min, beginning 200°C-min after fertilization, using a commercial system (model TRC-APVM, TRC Hydraulics Inc., Dieppe, NB, Canada). Untreated eggs were retained as diploid controls. Embryos and larvae were incubated in the dark until yolk-sac absorption in March 2019, after which the fry were moved to circular flow-through tanks with dechlorinated municipal water at ambient temperature and reared using standard salmonid culture protocols (Jobling *et al.*, 2010).

Fish from four stock tanks (two per ploidy) were visually size matched and transferred into 56L tanks in two identical 18-tank recirculating systems in separate rooms (9 tanks per ploidy per system, with 20 fish per tank). The systems were supplied with dechlorinated municipal water with a flow rate of 0.1L/min in each tank. Ploidy was randomly assigned to each tank before the fish were moved in. Fish were kept on a seasonally adjusted photoperiod for the duration of the experiment and fed 1% tank biomass of 2mm salmonid pellets (Corey Feed Mills Ltd., Fredericton, NB, Canada) by hand once daily between 9 and 11AM leading up to the trials, and then 0.5% biomass per day while trials were ongoing. Oxygen levels in the tanks were kept as close to 100% air

saturation as possible; the minimum value measured was 92%. After a month in these tanks, the fish were anesthetized (1% tert-amyl alcohol; catalogue number A730-1, Thermo Fisher, Ottawa, ON, Canada), measured (body mass [g] and fork length [cm]) and fin-clipped for identification during trials (left and right pectoral clips for fish assigned to the 15 and 18°C acclimation groups, respectively). Target acclimation temperatures were then achieved by increasing or decreasing the temperature of the two tank systems by 1°C per day, after which fish were habituated to their respective acclimation temperature for two weeks. Temperatures and dissolved oxygen levels in each tank were measured daily using a handheld oxygen meter (model ProODO™, YSI Inc., Yellow Springs, OH, USA) between 8 and 11AM.

2.1.2 Experimental Trials

One trial was carried out per day for 35 consecutive days. Each trial began between 8:30 and 9:30AM, with fish fasted for 24 hours before their trial. Fish were selected for each trial by randomly assigning a home tank to a trial day and then haphazardly selecting three diploid and three triploid fish from each temperature acclimation group to be placed in the experimental arena (i.e., 12 fish per trial on any given day). Trials were conducted at one of seven randomly assigned temperatures (20, 22, 24, 26, 28, 30°C or the ambient temperature of the incoming water to the aquatic facility [average 15.3°C; range 14.2-15.7°C]). A total of 15 fish of each ploidy and acclimation temperature were tested in this way (420 fish in total).

The experimental arena was a 17L stainless steel water bath (model A10B, Thermo Fisher) containing 15L of dechlorinated water from the same source as the water

entering the home tanks. A bubble diffuser attached to compressed air was used to keep dissolved oxygen at 100% air saturation; this was monitored using the dissolved oxygen meter. The water bath also contained a plastic net to prevent fish from entering the pump area and had a corrugated plastic wall around it to prevent fish from jumping out. The immersion heater/circulator (model SC100, Thermo Fisher) was then turned on to heat the water to the desired experimental temperature at an average rate of 0.4°C/min. The fish were then held for one hour at this trial temperature, after which the air bubble diffuser was replaced by a separate bubble diffuser used to inject compressed nitrogen into the water to displace oxygen.

Oxygen depletion was maintained manually at a rate of 0.75% decrease/min by adjusting the valve on the nitrogen cylinder, using the dissolved oxygen meter to monitor levels. As each individual fish lost equilibrium, temperature and dissolved oxygen (as % air saturation) were recorded and it was moved using a dip net to a separate aerated recovery tank containing 1L of dechlorinated water at the fish's starting temperature. "Loss of equilibrium" was defined as the fish floating belly up in the water bath without righting itself for five seconds. Dissolved oxygen data were later converted to oxygen tension (PO_2; kPa) using open-access software (PreSens Oxygen Unit Calculation; https:// www.presens.de/support-services/download-center/tools-utilities.html).

After all fish within a trial had lost equilibrium, they were individually anaesthetized and measured (as above) and assigned to acclimation group based on their fin clip. Then, using a heparinized needle and syringe, blood was taken from the caudal vasculature, transferred to a 0.5mL microcentrifuge tube and frozen for later hemoglobin

analysis. A small amount of remaining blood was used to make a blood smear for ploidy confirmation. Fish were then euthanized by overdose in 10% tert-amyl alcohol.

2.1.3 Dissection

Fish were dissected as soon as possible after euthanasia. The second gill arch on the left side was first excised and transferred to 10% buffered formalin (catalogue number SF99-4; Thermo Fisher) for fixation prior to future histological preparation for determining interlamellar cell mass (ILCM) size. The fish was then cut from vent to operculum and the whole heart was excised. The excess tissue was trimmed, and then the ventricle was weighed to determine relative ventricular mass (RVM; Formula 1) and transferred to Bouin's solution (catalogue number HT10132, Sigma-Aldrich, St. Louis, MO, USA) for fixation for future histological preparation for determining the ratio of spongy to compact myocardium. The liver was also excised and weighed to determine hepatosomatic index (HSI; Formula 2). Condition factor, which is a unitless measure of a fish's mass-to-length ratio, was calculated as per Formula 3.

Formula 1: $\text{RVM} = \frac{Ventricle\ mass\ (g)}{Body\ mass\ (g)}\ X\ 100$

Formula 2: $\text{HSI} = \frac{Liver\ mass\ (g)}{Body\ mass\ (g)}\ X\ 100$

Formula 3: $\text{Condition Factor} = \frac{10^2 \times Body\ mass\ (g)}{Fork\ length\ (cm)^3}$

2.1.4 Ploidy Confirmation

Ploidy was confirmed by imaging the blood smears at 40X using a camera attachment (model MU900, AmScope, Irvine, CA, USA) connected via USB to a computer with imaging software (AmScope MU series for Windows version 3.7). Five

fields of view from each slide were imaged and the lengths of five red blood cells from each of these fields measured for the calculation of average red blood cell length, a technique validated by Benfey *et al.* (1984) based on ploidy-dependent increase in red blood cell size.

2.1.5 Hematology

Blood samples were thawed in room temperature water prior to hemoglobin measurement via the cyanmethemoglobin method using Drabkin's reagent (catalogue number 2660-16, Thermo Fisher) and human hemoglobin as standards (catalogue number H7379, Sigma-Aldrich). Each sample was measured in duplicate.

2.1.6 Statistical Analyses

All statistical analyses were performed in R version 3.5.1 (R Core Team, 2019). Separate general linear models (GLMs) were first used to determine if ploidy or acclimation temperature affected body mass, fork length, condition factor, RVM, HSI, or blood hemoglobin concentration. Two separate GLMs with full interactions among all variables were then performed; one for PO_2 at loss of equilibrium and the other for time to loss of equilibrium. The variables tested were ploidy (2 levels), acclimation temperature (2 levels), test temperature (7 levels), RVM (continuous), condition factor (continuous), HSI (continuous), and blood hemoglobin concentration (continuous). A test of assumptions showed that the data did not fit the assumption of homoscedasticity, and therefore a Box-Cox square-root transformation was applied to both the PO_2 and time to loss of equilibrium data. Statistically insignificant ($p>0.05$) interactions were removed and the model run again until all interactions and the main effects were significant,

otherwise known as the hypothesis testing method of model building (Zuur *et al*., 2009). Tukey's *post-hoc* tests were performed after the GLM, using test temperature as the variable of interest. Fish that failed to complete the entire experimental protocol (i.e., did not last the entire hour at their respective test temperatures) were included in the analyses summarized below. Separate analyses that excluded them (not shown) did not affect any of the outcomes with respect to which factors had a significant effect on the response variables of interest.

2.2 Experiment 2

The second experiment used the same methods as the first with the following exceptions:

2.2.1 Rearing Information

Fish from four stock tanks (two per ploidy) were visually size matched and transferred into 20 treatment tanks (5 per ploidy per recirculating system, with 20 fish per tank), instead of 36 treatment tanks.

Fish were then acclimated to their randomly assigned temperature (either 15 or 18°C) for four full weeks. Over the course of three days, the temperature of the tanks at 18°C was then reduced to 15°C by 1°C per day, and all fish were then held at 15°C for an additional 10 days before trials started.

2.2.2 Experimental Trials

Each experimental trial was carried out on a different day over 21 consecutive days, with the result that the time interval between reacclimation of warm-acclimated fish to 15°C and testing them increased by a day with each trial. The experimental

temperature was either 24, 26, 28, 30°C or the ambient temperature of the incoming water to the aquatic facility (average 14.4°C; range 14.1-15.1°C). These temperatures were randomly assigned to trial days. There were 4 trials at each elevated temperature and 5 at ambient temperature.

2.2.3 Hematology

Hematocrit was measured as an alternative to total blood hemoglobin. Blood was collected immediately after the trial and two pre-heparinized capillary tubes were filled for each fish. These were centrifuged for 10 minutes (Model MB, International Equipment Company), and then total blood height and red blood cell height were measured. Hematocrit was calculated as per Formula 4.

Formula 4: Hematocrit = $\frac{RBC\ height\ (cm)}{Total\ height\ (cm)}\ x\ 100\%$

2.2.4 Statistical Analyses

Identical GLMs were used as for the previous experiment, except that hematocrit replaced blood hemoglobin concentration, test temperature had 5 levels, and days since reacclimation of warm-acclimated fish to 15°C (hereafter referred to as "days out of acclimation"; 20 levels) was included as an additional variable. A Box-Cox transformation was applied to the PO_2 at loss of equilibrium data because they again failed to meet the assumption of homoscedasticity. Fish that failed to complete the entire experimental protocol were again included in the analyses; excluding them had no effect on any of the outcomes.

3. Results

3.1 Experiment 1

All fish were confirmed to be of the correct ploidy, with all presumed diploids and triploids having average erythrocyte lengths of less than or greater than 200 pixels, respectively (Figure 1).

Fork length and condition factor (but not body mass) were significantly different between ploidies and acclimation temperatures (Table 1). Triploids were longer and had a lower condition factor than diploids, and fish acclimated to 15°C were longer and had a lower condition factor than those acclimated to 18°C (Figure 2). Acclimation temperature affected RVM but not blood hemoglobin concentration, and ploidy affected blood hemoglobin concentration but not RVM (Table 1). Fish acclimated to 18°C had lower RVM than those acclimated to 15°C and triploids had lower blood hemoglobin concentration that diploids (Figure 3). HSI was not affected by ploidy or acclimation temperature (Table 1; Figure 3).

Ploidy, acclimation temperature, test temperature and the interaction between acclimation temperature and test temperature all had significant effects on the PO_2 at loss of equilibrium and time to loss of equilibrium (Tables 2 & 3). Triploids had a higher PO_2 at loss of equilibrium and shorter time to loss of equilibrium when compared to diploids of the same temperature acclimation group and fish acclimated to 18°C had a lower PO_2 at loss of equilibrium and a longer time to loss of equilibrium than those acclimated to 15°C regardless of ploidy (Figures 4, 5, 6 & 7).

A post-hoc Tukey test revealed that the PO_2 at loss of equilibrium did not differ among test temperatures 20, 22, and 24°C, but was then significantly higher with each successive increase in temperature.

PO_2 at loss of equilibrium and time to loss of equilibrium were both affected by HSI (Tables 2 and 3). Fish with a higher HSI tended to have a higher PO_2 at loss of equilibrium (Figure 8) and shorter time to loss of equilibrium (Figure 9). Time to loss of equilibrium was also affected by condition factor (Table 3), tending to be shorter in fish with higher condition factor (Figure 10). PO_2 at loss of equilibrium and time to loss of equilibrium were not affected by RVM or blood hemoglobin concentration (Tables 2 and 3).

3.2 Experiment 2

All fish were confirmed to be of the correct ploidy, with presumptive diploids and triploids having erythrocyte length of less than or greater than 200 pixels, respectively (Figure 11).

Fork length and condition factor (but not body mass) were significantly different between previous acclimation temperature groups, and condition factor was also significantly different between ploidies (Table 4). Fish previously acclimated to 18°C were longer and had a lower condition factor than those reared at 15°C, and triploids had a lower condition factor than diploids (Figure 12). Previous acclimation temperature affected RVM and HSI, and ploidy affected HSI as well (Table 4). Fish previously acclimated to 18°C had a higher RVM and lower HSI than those previously acclimated to

15°C, and triploids had a lower HSI than diploids (Figure 13). Hematocrit was not affected by ploidy or previous acclimation temperature (Table 4; Figure 13).

Ploidy affected time to loss of equilibrium but not PO_2 at loss of equilibrium, whereas both endpoints were affected by previous acclimation temperature, test temperature and the number of days out of acclimation (Tables 5 & 6). Triploids had a shorter time to loss of equilibrium than diploids, and PO_2 at loss of equilibrium was higher, and time to loss of equilibrium shorter, for fish previously acclimated to 18°C, for fish tested at higher temperatures, and for fish out of acclimation for a longer time before testing (Figures 14-19). Post-hoc Tukey tests revealed significant differences ($p<0.0001$) among all test temperatures except for the 28 and 30°C test temperatures for PO_2 at loss of equilibrium, and among all test temperatures for time to loss of equilibrium.

PO_2 at loss of equilibrium and time to loss of equilibrium were affected by both HSI and hematocrit, and time to loss of equilibrium was also affected by condition factor (Table 5 & 6). PO_2 at loss of equilibrium tended to be higher, and time to loss of equilibrium shorter, in fish with higher HSIs and lower hematocrits, and time to loss of equilibrium also tended to be higher in fish with higher conditions factors (Figures 20-24). PO_2 at loss of equilibrium and time to loss of equilibrium were not affected by RVM (Tables 5 & 6).

Table 1: Results of General Linear Models testing the effects of ploidy, acclimation temperature, and their interactions on body mass, fork length, condition factor, relative ventricular mass, hepatosomatic index, and blood hemoglobin concentration of juvenile brook charr (*Salvelinus fontinalis*). Bold values indicate a significant relationship ($p<0.05$).

Source of Variation	df	MS	F-value	P-value
Body mass				
Ploidy	1	24.82	2.19	0.14
Acclimation Temp	1	19.15	1.69	0.20
Ploidy x Acclimation Temp	1	7.48	0.66	0.42
Residuals	416	11.36		
Fork length				
Ploidy	**1**	**10.18**	**9.75**	**0.0019**
Acclimation Temp	**1**	**7.61**	**7.29**	**0.0072**
Ploidy x Acclimation Temp	1	0.51	0.49	0.49
Residuals	416	1.04		
Condition Factor				
Ploidy	**1**	**0.35**	**45.41**	**5.34×10^{-11}**
Acclimation Temp	**1**	**0.23**	**30.46**	**6.01×10^{-8}**
Ploidy x Acclimation Temp	1	0.012	1.5	0.22
Residuals	416	0.0077		
Relative Ventricular Mass				
Ploidy	1	0.000001	0.0094	0.92
Acclimation Temp	**1**	**0.00575**	**4.770**	**0.030**
Ploidy x Acclimation Temp	1	0.000348	0.2890	0.59
Residuals	416	0.00121		
Hepatosomatic Index				
Ploidy	1	0.0337	0.2469	0.62
Acclimation Temp	1	0.166	1.2180	0.27
Ploidy x Acclimation Temp	1	0.165	1.2095	0.27
Residuals	416	0.136		
Hemoglobin				
Ploidy	**1**	**124.702**	**4.1225**	**0.043**
Acclimation Temp	1	41.112	1.3591	0.24
Ploidy x Acclimation Temp	1	2.585	0.0854	0.77
Residuals	416	30.249		

MS=Mean Squares

Table 2: Results of a General Linear Model testing the effects of ploidy, acclimation temperature, test temperature, condition factor, relative ventricular mass, hepatosomatic index, and blood hemoglobin content on PO_2 at loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*). The model was created with non-statistically significant interactions ($p>0.05$) with and between covariates and main effects removed. A square-root transformation was applied to the PO_2 at loss of equilibrium data. Bold values indicate a significant relationship ($p<0.05$).

Source of Variation	df	MS	F- value	p-value
Ploidy	**1**	**0.283**	**9.542**	**0.0022**
Acclimation Temperature	**1**	**5.955**	**200.838**	**$<2.2 \times 10^{-16}$**
Test Temperature	**6**	**55.959**	**1887.356**	**$<2.2 \times 10^{-16}$**
Condition Factor	1	0.0001	0.0159	0.90
Relative Ventricular Mass	1	0.001	0.0419	0.84
Hepatosomatic Index	**1**	**0.748**	**25.225**	**7.70×10^{-7}**
Hemoglobin	1	0.044	1.500	0.22
Acclimation Temp x Test Temp	**6**	**1.302**	**43.909**	**$<2.2 \times 10^{-16}$**
Residuals	400	0.030		

MS=Mean Squares

Table 3: Results of a General Linear Model testing the effects of ploidy, acclimation temperature, test temperature, condition factor, relative ventricular mass, hepatosomatic index and blood hemoglobin content on time to loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*). The model was created with non-statistically significant interactions ($p>0.05$) with and between covariates and main effects removed. A square-root transformation was applied to the time at loss of equilibrium data. Bold values indicate a significant relationship ($p<0.05$).

Source of Variation	df	MS	F- value	p-value
Ploidy	**1**	**0.89**	**5.534**	**0.019**
Acclimation Temperature	**1**	**46.38**	**286.832**	**$<2.2 \times 10^{-16}$**
Test Temperature	**6**	**347.57**	**2149.355**	**$<2.2 \times 10^{-16}$**
Condition Factor	**1**	**1.12**	**6.951**	**0.0087**
Relative Ventricular Mass	1	0.03	0.168	0.68
Hepatosomatic Index	**1**	**1.02**	**6.301**	**0.012**
Hemoglobin	1	0.0001	0.0077	0.93
Acclimation Temp x Test Temp	**6**	**15.38**	**95.124**	**$<2.2 \times 10^{-16}$**
Residuals	400	0.16		

MS=Mean Squares

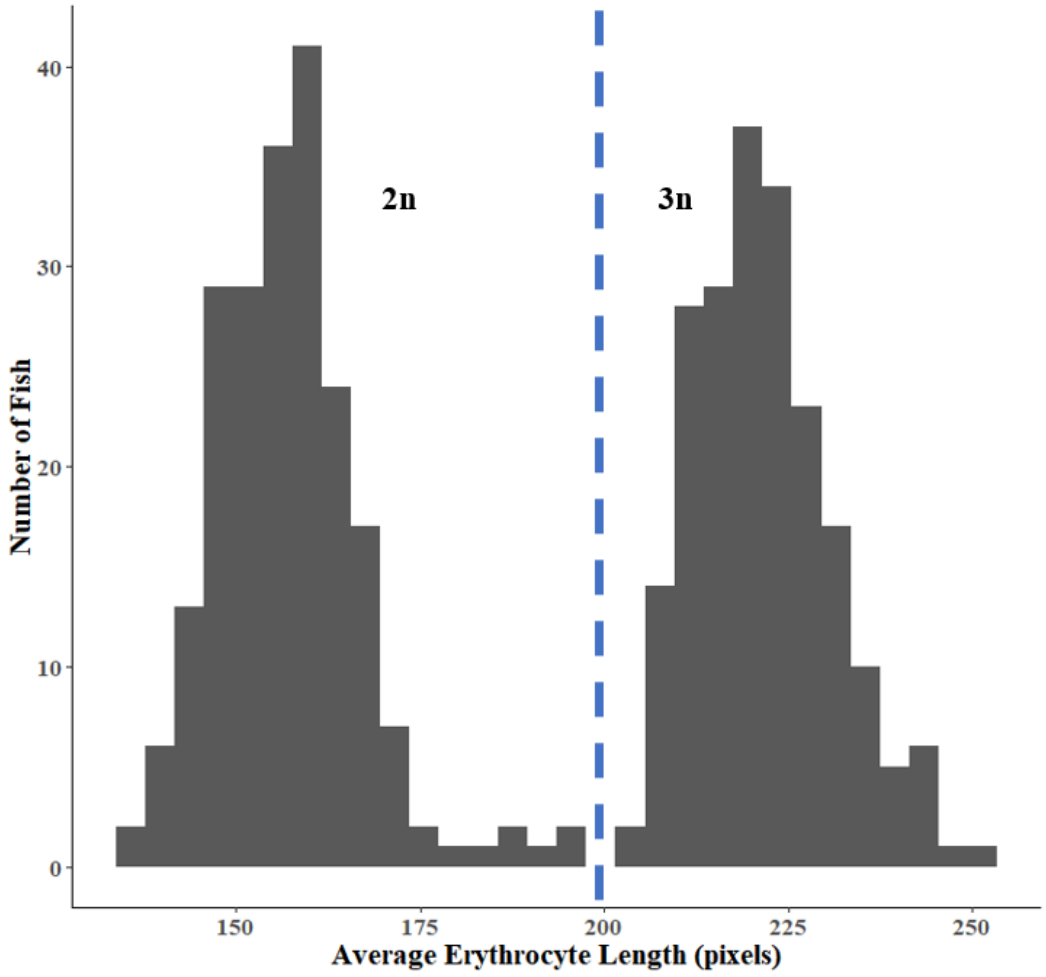

Figure 1: The average erythrocyte length for 210 diploid and 210 triploid juvenile brook charr (*Salvelinus fontinalis*) used to determine the effect of acclimation temperature on hypoxia tolerance. Dashed line represents the separation between putative ploidies based on whether they were derived from control (left) or pressure-treated (right) eggs.

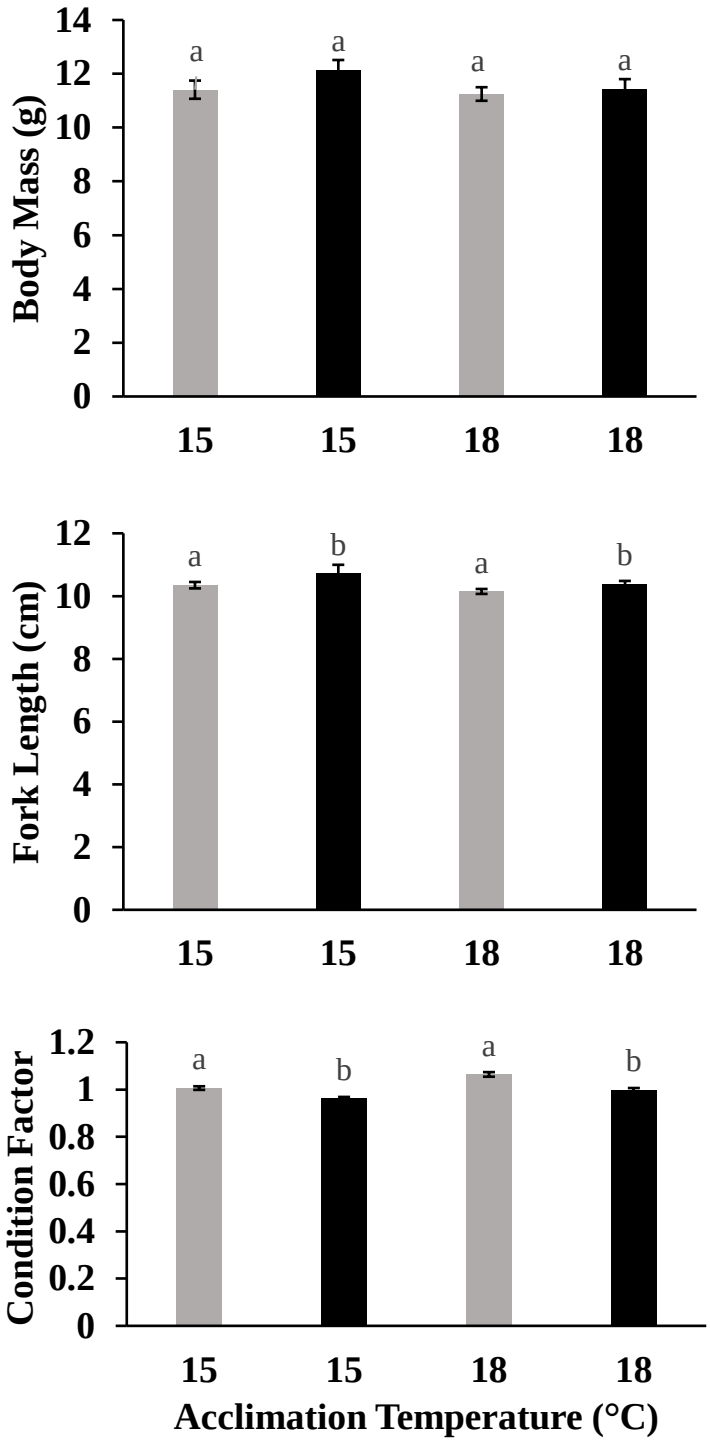

Figure 2: The effect of ploidy (diploid in light grey and triploid in dark grey) and acclimation temperature on body mass, fork length, and condition factor in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; sample size of 105 for each group). Letters over bars indicate significant differences between groups ($p<0.05$).

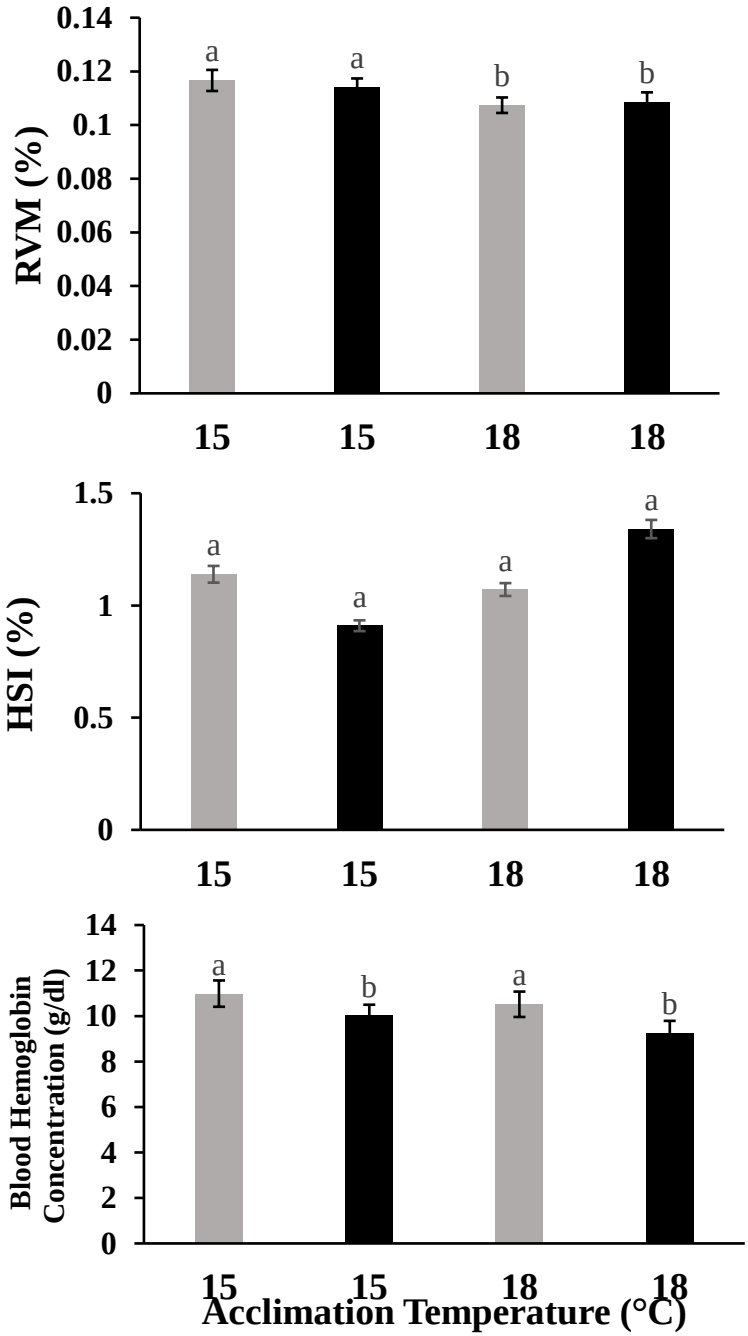

Figure 3: The effect of ploidy (diploid in light grey and triploid in dark grey) and acclimation temperature on relative ventricular mass (RVM), hepatosomatic index (HSI), and blood hemoglobin concentration in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; sample size of 105 for each group). Letters over bars indicate significant differences between groups ($p<0.05$).

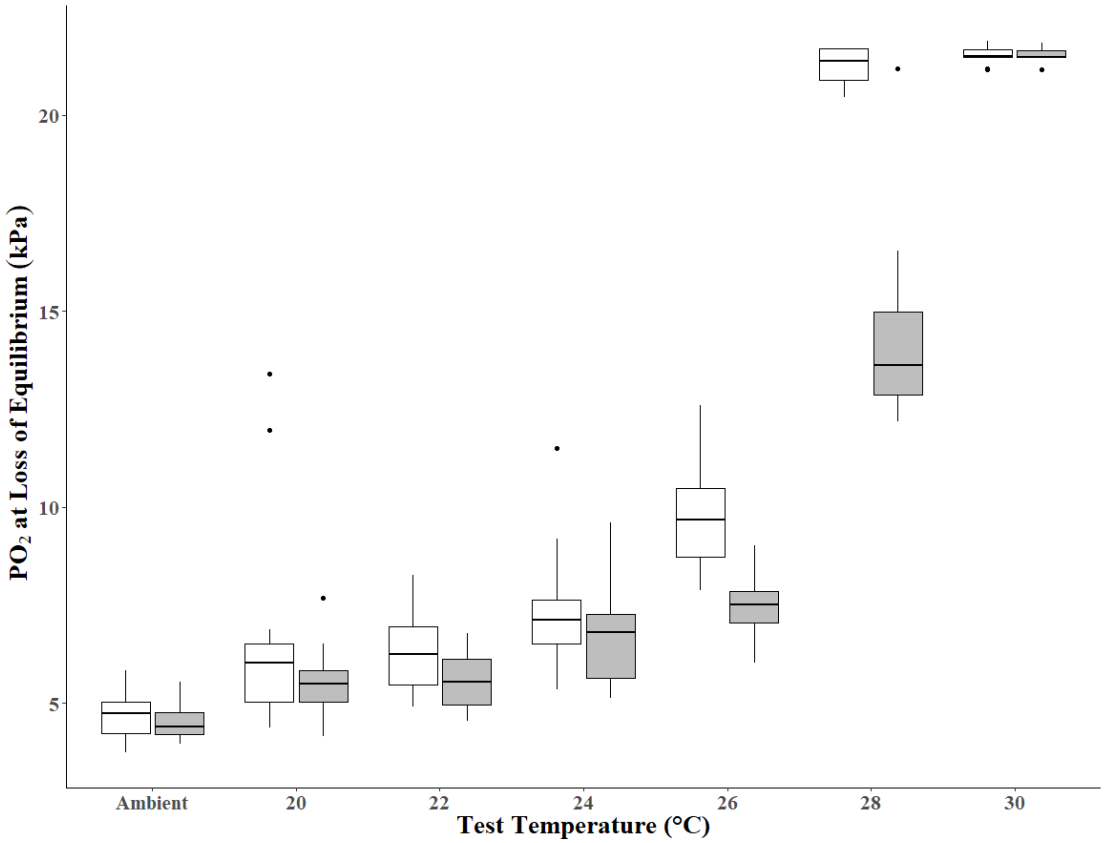

Figure 4: Oxygen tension (PO_2) at loss of equilibrium of pooled diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures separated by temperature acclimation group (15 and 18°C in white and light grey, respectively; n=30 for each boxplot). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

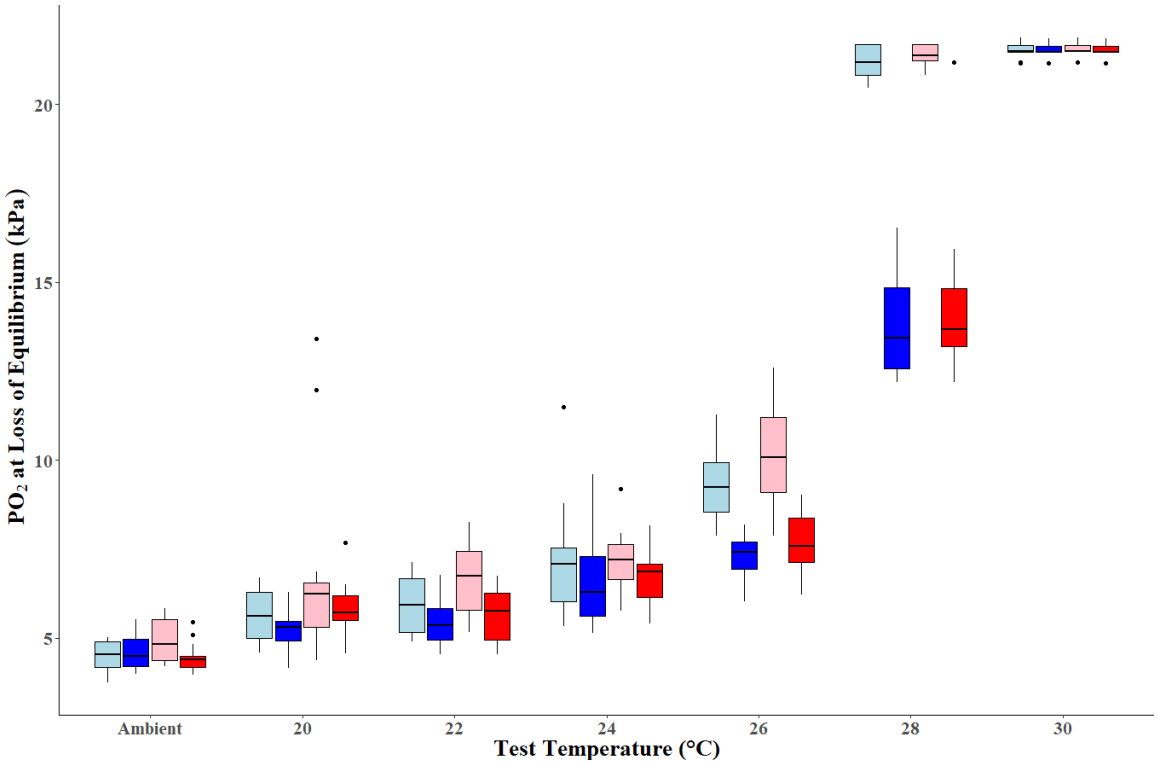

Figure 5: Oxygen tension (PO_2) at loss of equilibrium of juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures separated by ploidy and temperature acclimation group (diploids at 15°C and 18°C in light and dark blue, respectively, triploids at 15°C and 18°C in pink and red, respectively; n=15 for each boxplot). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

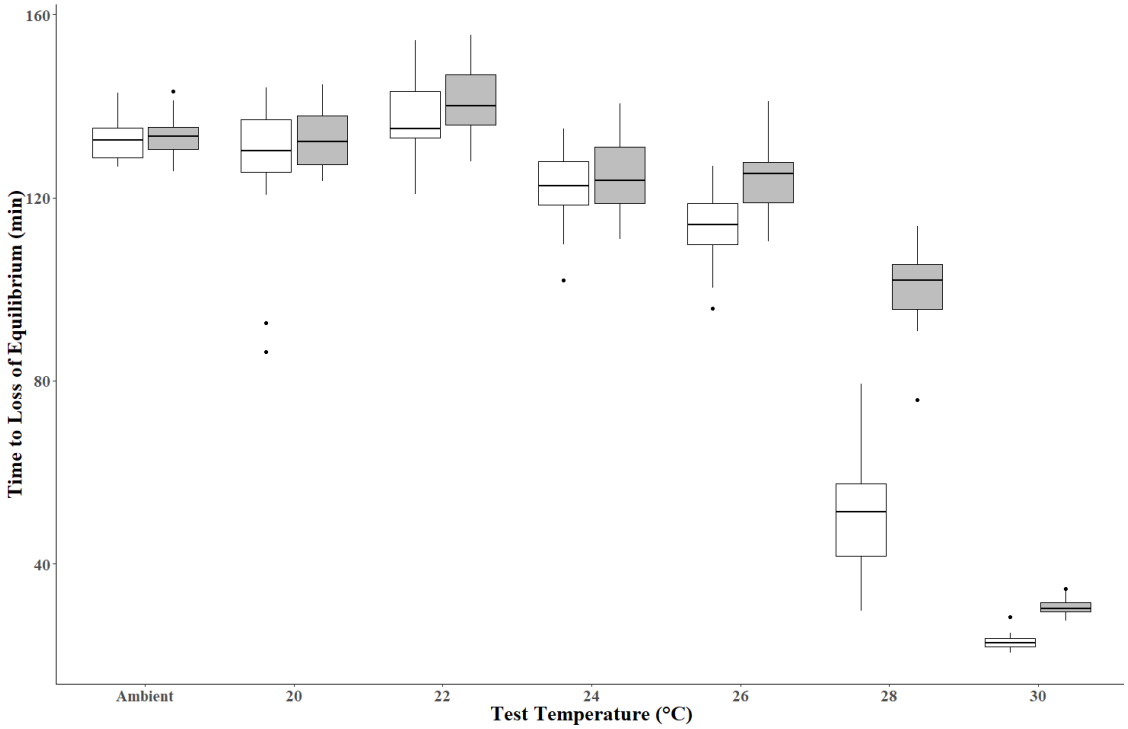

Figure 6: Time to loss of equilibrium of pooled diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures separated by temperature acclimation group (15 and 18°C in white and light grey, respectively; n=30 for each boxplot). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

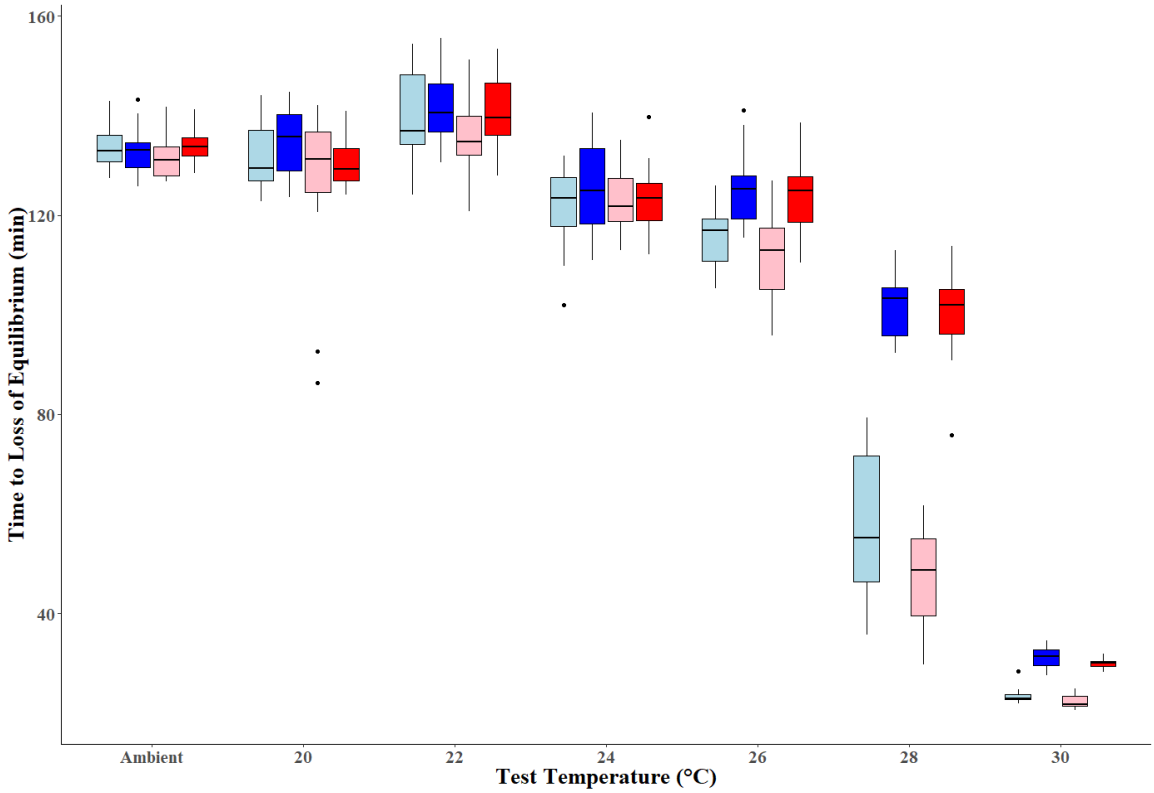

Figure 7: Time loss to equilibrium of juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures separated by ploidy and temperature acclimation group (diploids at 15°C and 18°C in light and dark blue, respectively, triploids at 15°C and 18°C in pink and red, respectively; n=15 for each boxplot). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

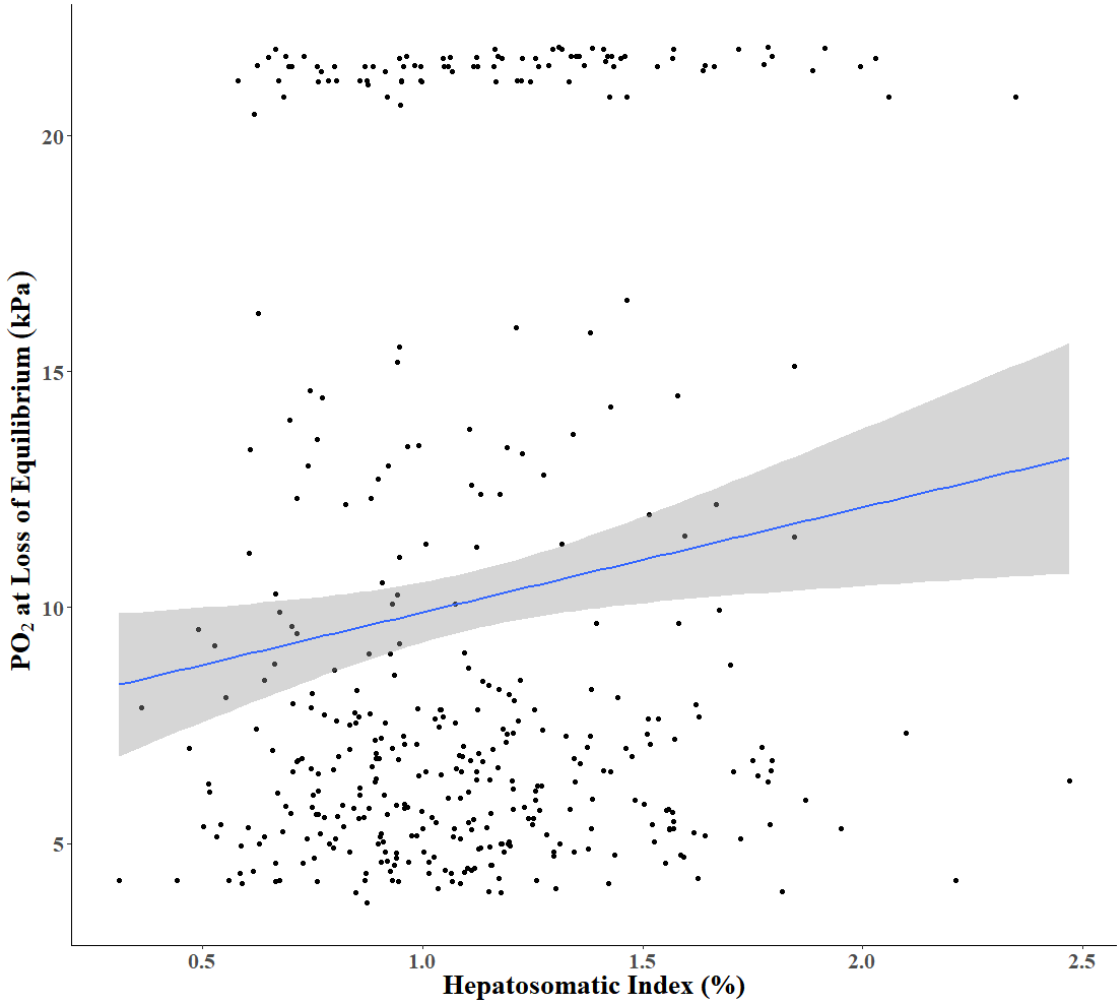

Figure 8: The effect of hepatosomatic index on PO_2 at loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures (ambient, 20, 22, 24, 26, 28 and 30°C; n=420). Blue line represents linear regression, grey area is 95% confidence interval.

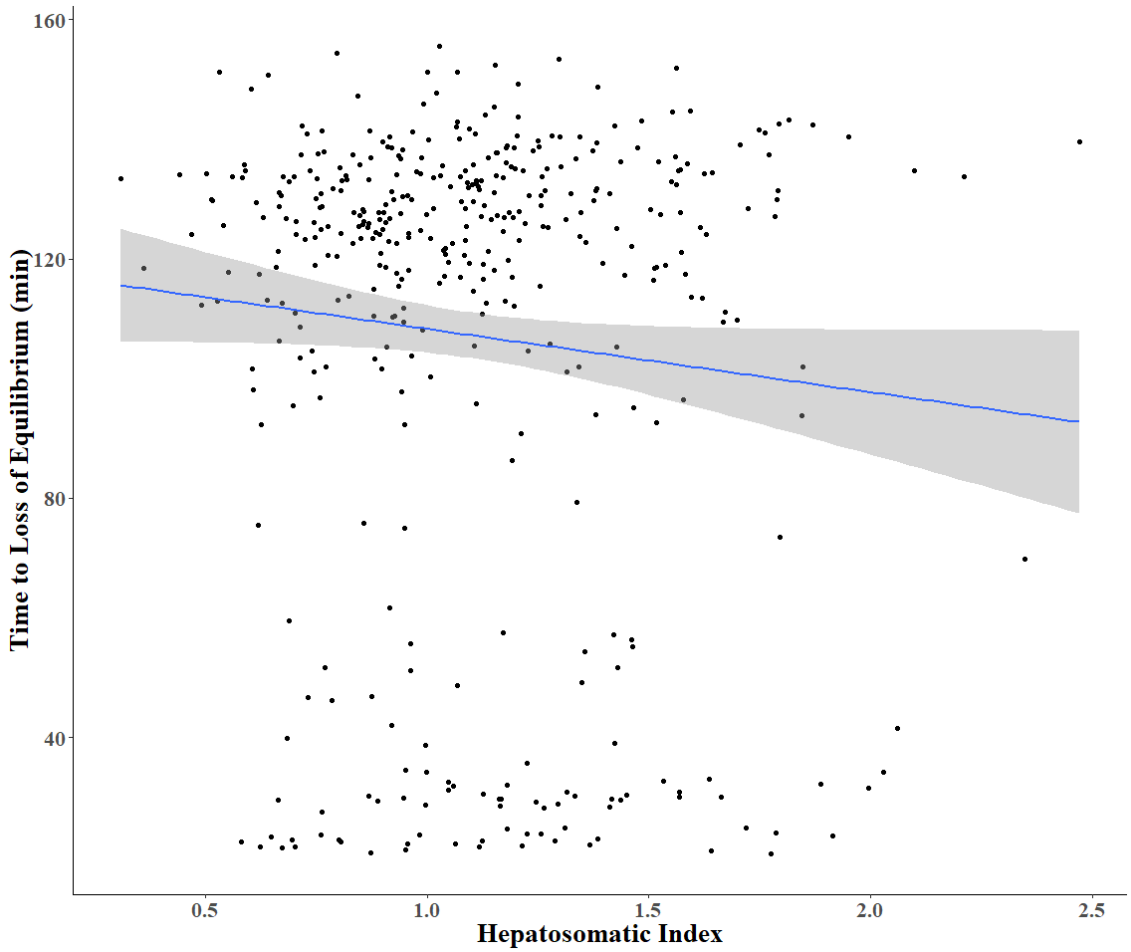

Figure 9: The effect of hepatosomatic index on time to loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures (ambient, 20, 22, 24, 26, 28 and 30°C; n=420). Blue line represents linear regression, grey area is 95% confidence interval.

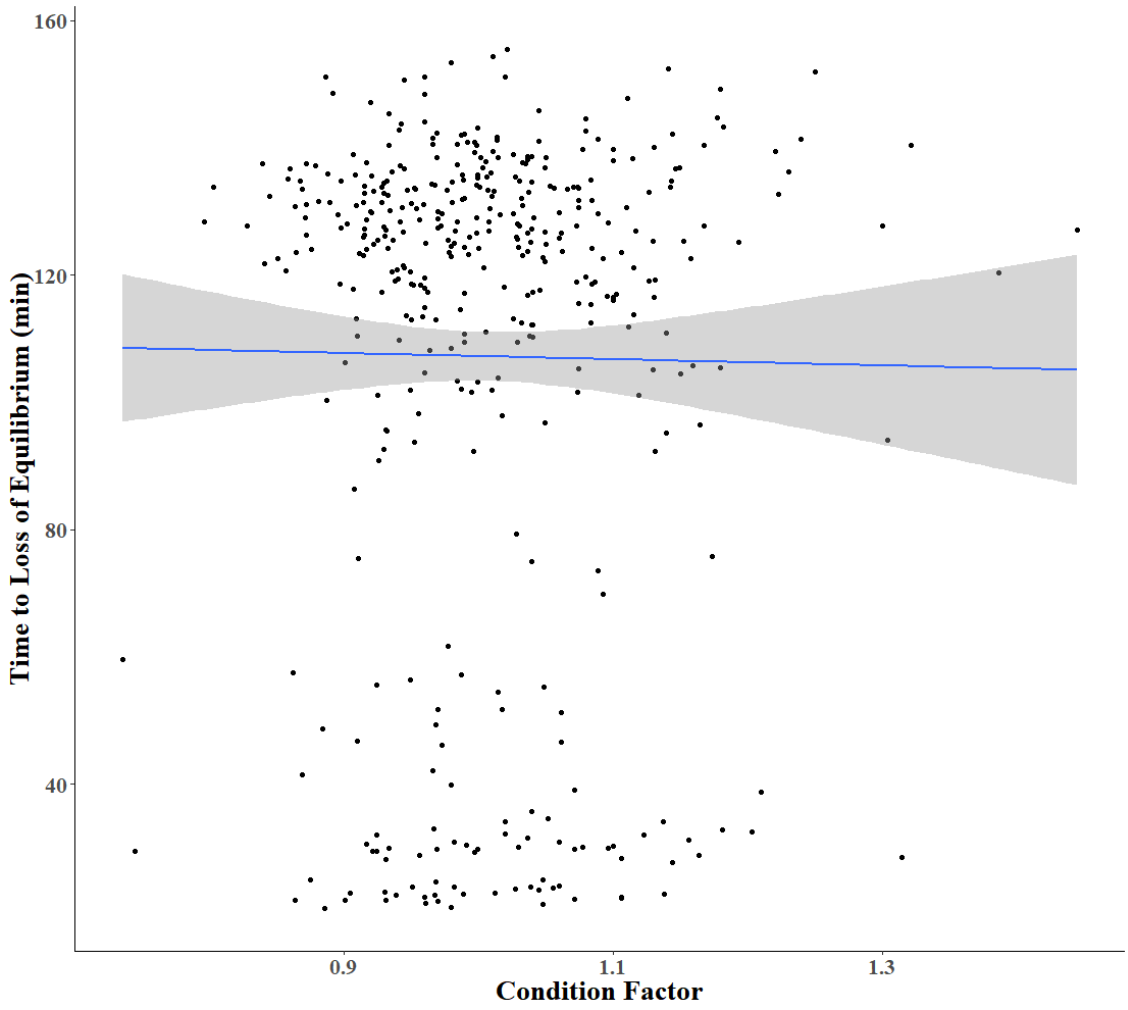

Figure 10: The effect of condition factor on time to loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at seven different test temperatures (ambient, 20, 22, 24, 26, 28 and 30°C; n=420). Blue line represents linear regression, grey area is 95% confidence interval.

Table 4: Results of General Linear Models testing the effects of ploidy, previous acclimation temperature, and their interactions on body mass, fork length, condition factor, relative ventricular mass, hepatosomatic index, and hematocrit of juvenile brook charr (*Salvelinus fontinalis*). Bold values indicate a significant relationship ($p<0.05$).

Source of Variation	df	MS	F-value	P-value
Body mass				
Ploidy	1	13.27	0.20	0.66
Acclimation Temp	1	153.52	2.28	0.13
Ploidy x Acclimation Temp	1	104.41	1.55	0.21
Residuals	248	67.39		
Fork length				
Ploidy	1	1.62	1.59	0.21
Acclimation Temp	**1**	**21.40**	**21.04**	**7.15×10^{-6}**
Ploidy x Acclimation Temp	1	0.19	0.18	0.67
Residuals	248	1.02		
Condition Factor				
Ploidy	**1**	**0.13**	**14.91**	**1.44×10^{-3}**
Acclimation Temp	**1**	**0.38**	**45.30**	**1.17×10^{-10}**
Ploidy x Acclimation Temp	1	0.019	2.13	0.15
Residuals	248	0.0085		
Relative Ventricular Mass				
Ploidy	1	0.00049	0.6750	0.41
Acclimation Temp	**1**	**0.0048**	**6.5768**	**0.011**
Ploidy x Acclimation Temp	1	4.90×10^{-5}	0.0067	0.93
Residuals	248	0.00073		
Hepatosomatic Index				
Ploidy	**1**	**1.45**	**6.3399**	**0.012**
Acclimation Temp	**1**	**2.82**	**12.3518**	**0.00052**
Ploidy x Acclimation Temp	1	0.74	3.2213	0.074
Residuals	248	0.23		
Hematocrit				
Ploidy	1	64.89	1.7558	0.19
Acclimation Temp	1	0.82	0.0221	0.88
Ploidy x Acclimation Temp	1	74.07	2.0039	0.16
Residuals	248	36.96		

MS=Mean Squares

Table 5: Results of a General Linear Model testing the effects of ploidy, previous acclimation temperature, test temperature, days out of acclimation, condition factor, relative ventricular mass, hepatosomatic index, and hematocrit on PO_2 at loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*). The model was created with non-statistically significant interactions ($p>0.05$) with and between covariates and main effects removed. A Box-Cox transformation was applied to the PO_2 at loss of equilibrium data. Bold values indicate a significant relationship ($p<0.05$).

Source of Variation	df	MS	F- value	p-value
Ploidy	1	320	3.248	0.073
Acclimation Temperature	**1**	**492**	**4.9946**	**0.026**
Test Temperature	**4**	**4214.99**	**4280.47**	**$<2.2 \times 10^{-16}$**
Days out of Acclimation	**16**	**346**	**3.516**	**1.21×10^{-5}**
Condition Factor	1	311	3.160	0.077
Relative Ventricular Mass	1	103	1.041	0.31
Hepatosomatic Index	**1**	**466**	**4.733**	**0.031**
Hematocrit	**1**	**323**	**3.248**	**0.071**
Residuals	225	98		

MS=Mean Squares

Table 6: Results of a General Linear Model testing the effects of ploidy, previous acclimation temperature, test temperature, days out of acclimation, condition factor, relative ventricular mass, hepatosomatic index and hematocrit on time to loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*). The model was created with non-statistically significant interactions ($p>0.05$) with and between covariates and main effects removed. Bold values indicate a significant relationship ($p<0.05$).

Source of Variation	df	MS	F- value	p-value
Ploidy	**1**	**385**	**7.8920**	**0.0054**
Acclimation Temperature	**1**	**554**	**11.374**	**0.00089**
Test Temperature	**4**	**135985**	**2785.2696**	**$<2.2 \times 10^{-16}$**
Days out of Acclimation	**16**	**499**	**10.2164**	**$<2.2 \times 10^{-16}$**
Condition Factor	**1**	**421**	**8.6146**	**0.0037**
Relative Ventricular Mass	1	11	0.2309	0.63
Hepatosomatic Index	**1**	**314**	**6.4281**	**0.012**
Hematocrit	**1**	**786**	**16.0903**	**8.22×10^{-5}**
Residuals	225	49		

MS=Mean Squares

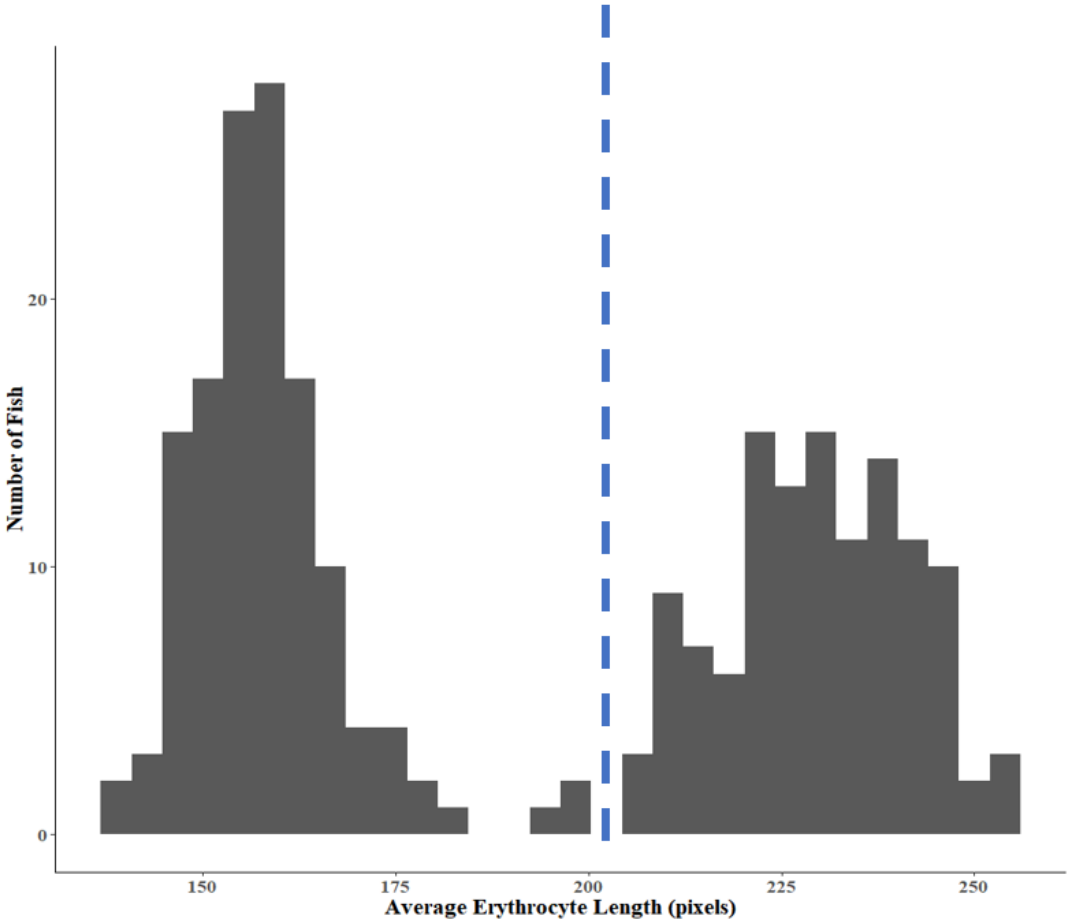

Figure 11: The average erythrocyte length for 126 diploid and 126 triploid juvenile brook charr (*Salvelinus fontinalis*) used to determine the long-term effect of previous acclimation temperature on hypoxia tolerance. Dashed line represents the separation between putative ploidies based on whether they were derived from control (left) or pressure-treated (right) eggs.

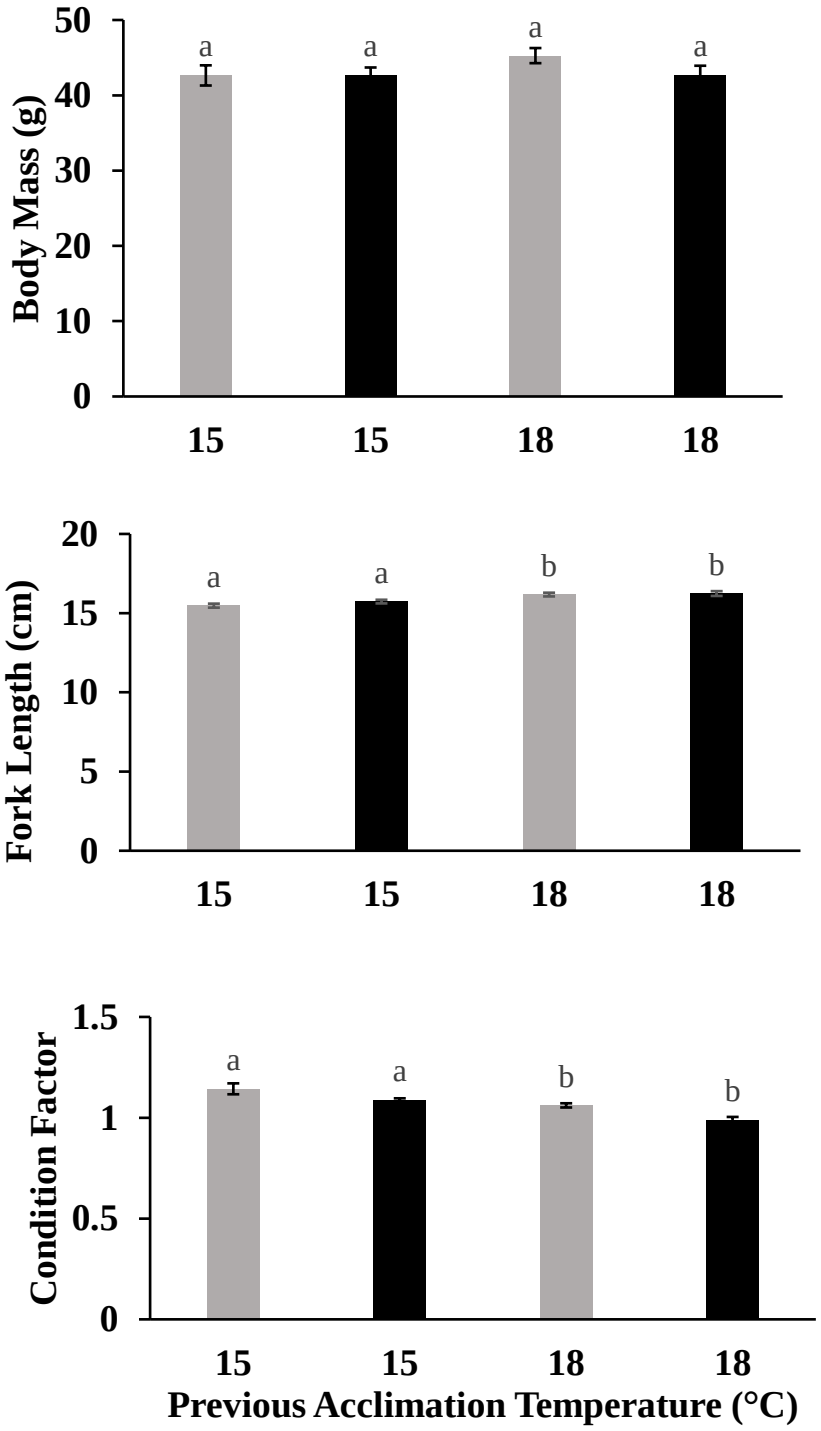

Figure 12: The effect of ploidy (diploid in light grey and triploid in dark grey) and previous acclimation temperature on body mass, fork length, and condition factor in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; sample size of 63 for each group). Letters over bars indicate significant differences between groups ($p<0.05$).

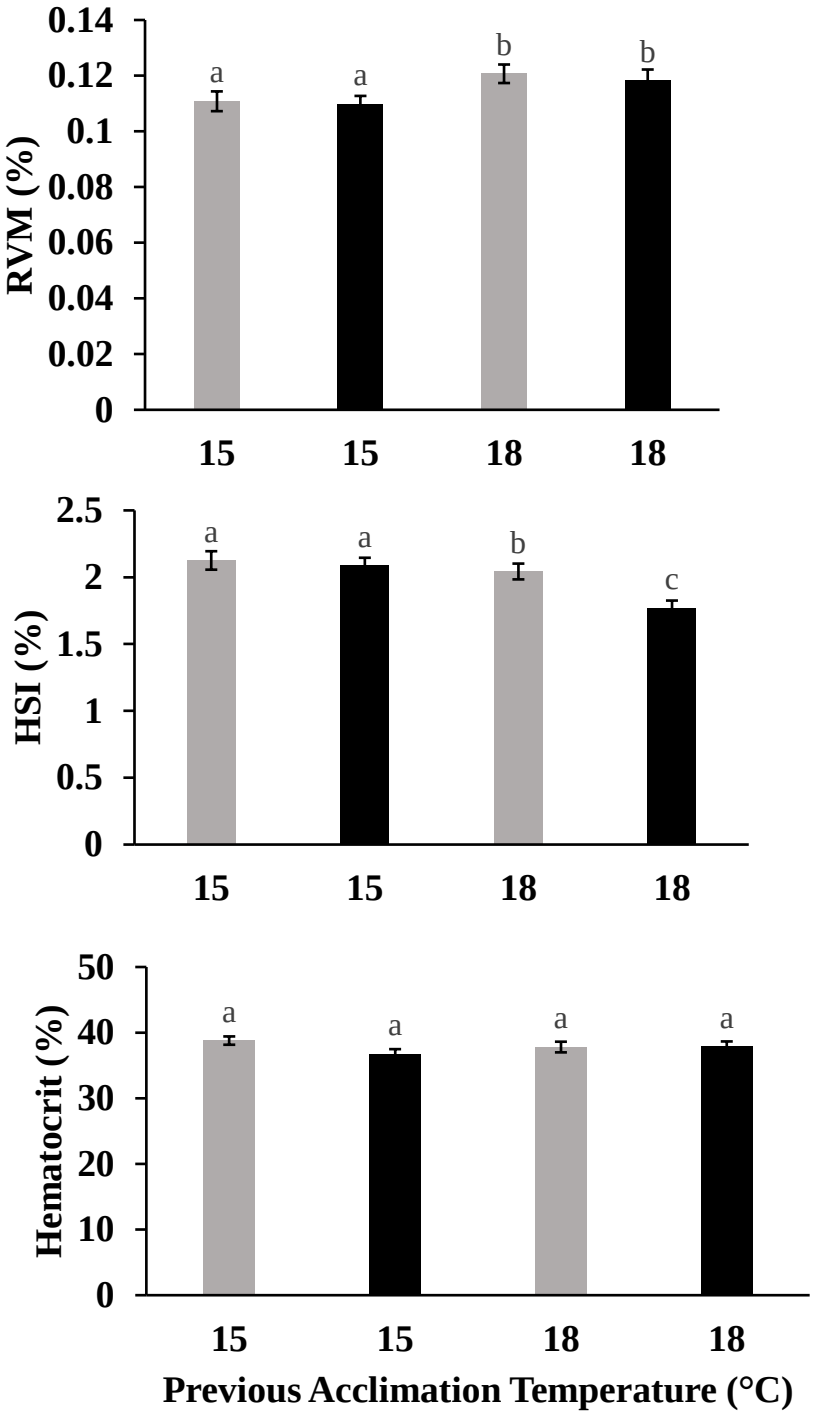

Figure 13: The effect of ploidy (diploid in light grey and triploid in dark grey) and previous acclimation temperature on relative ventricular mass (RVM), hepatosomatic index (HSI) and hematocrit in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; sample size of 63 for each group). Letters over bars indicate significant differences between groups ($p<0.05$).

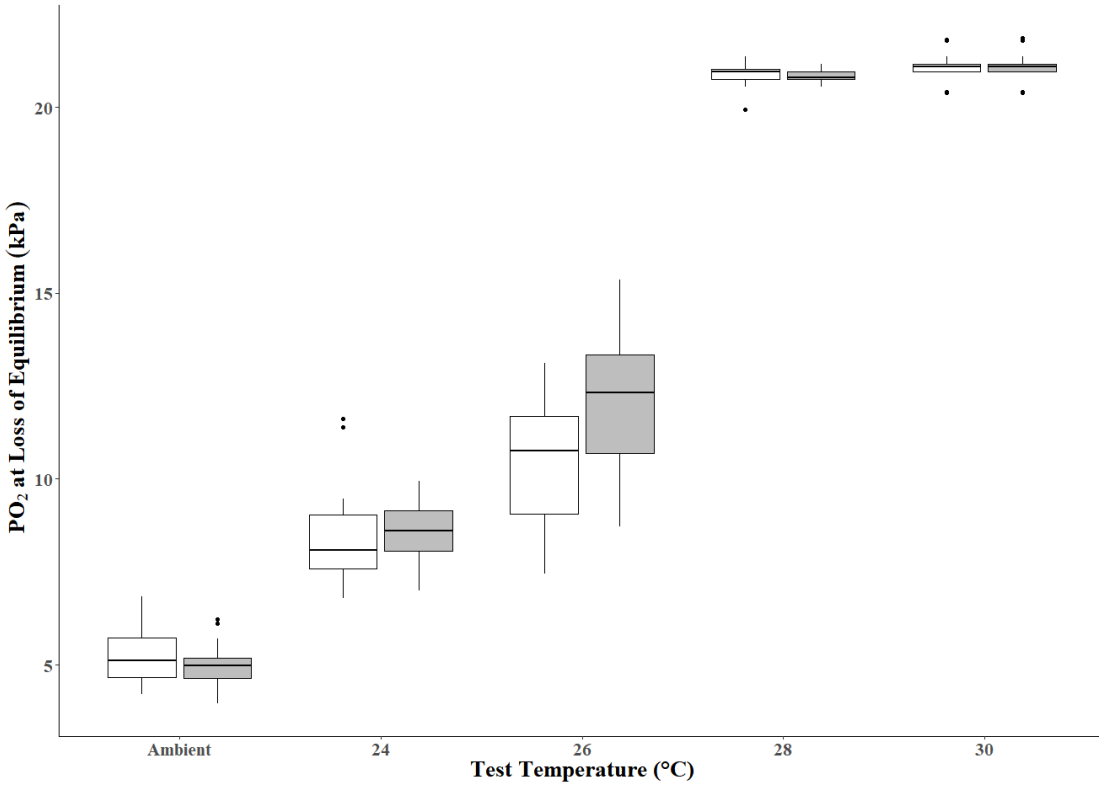

Figure 14: Oxygen tension (PO_2) at loss of equilibrium of pooled diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures separated by previous temperature acclimation group (15 and 18°C in white and light grey, respectively; n=24 for each boxplot, with the exception of the ambient temperature having 30 per box). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

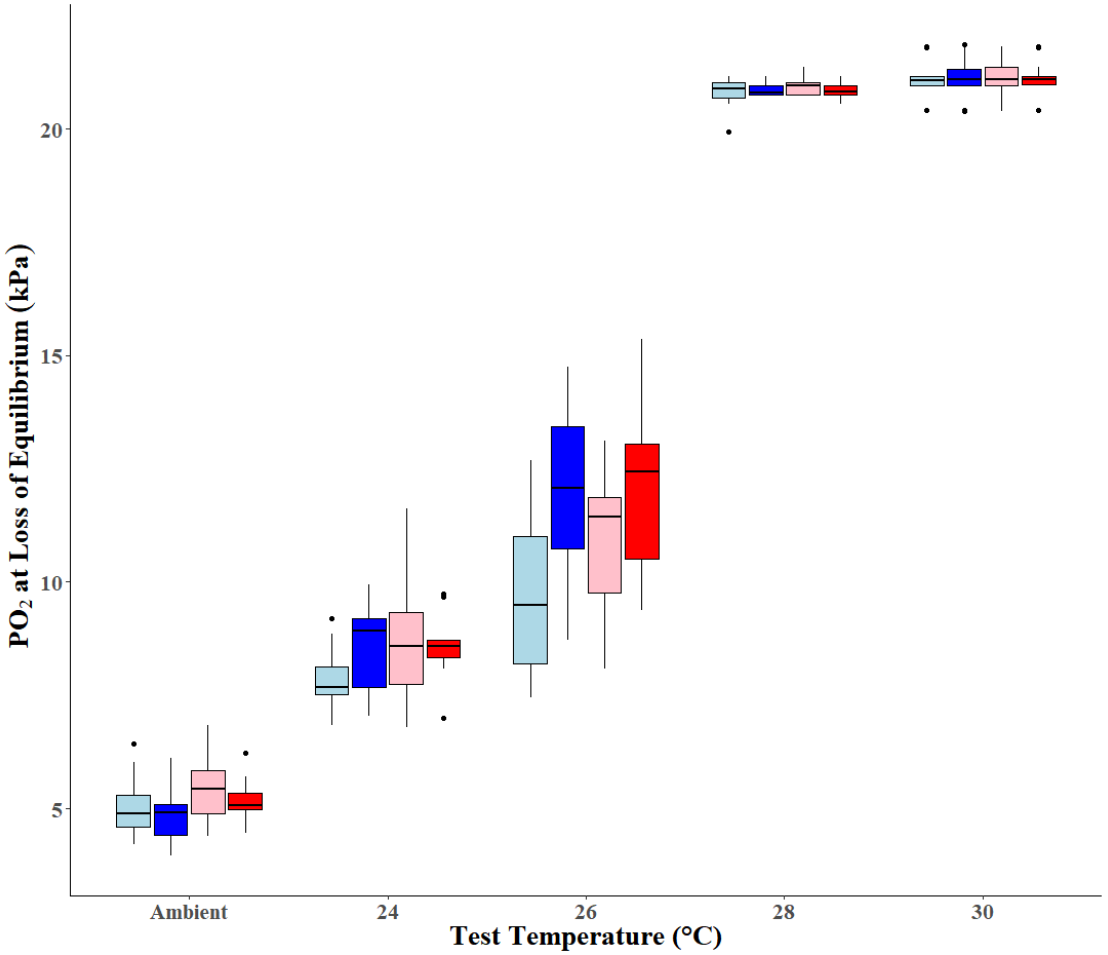

Figure 15: Oxygen tension (PO_2) at loss of equilibrium of juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures, separated by ploidy and previous temperature acclimation group (diploids at 15 and 18°C in light and dark blue, respectively, triploids at 15 and 18°C in pink and red, respectively; n=12 for each boxplot, with the exception of ambient boxes having n=15). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

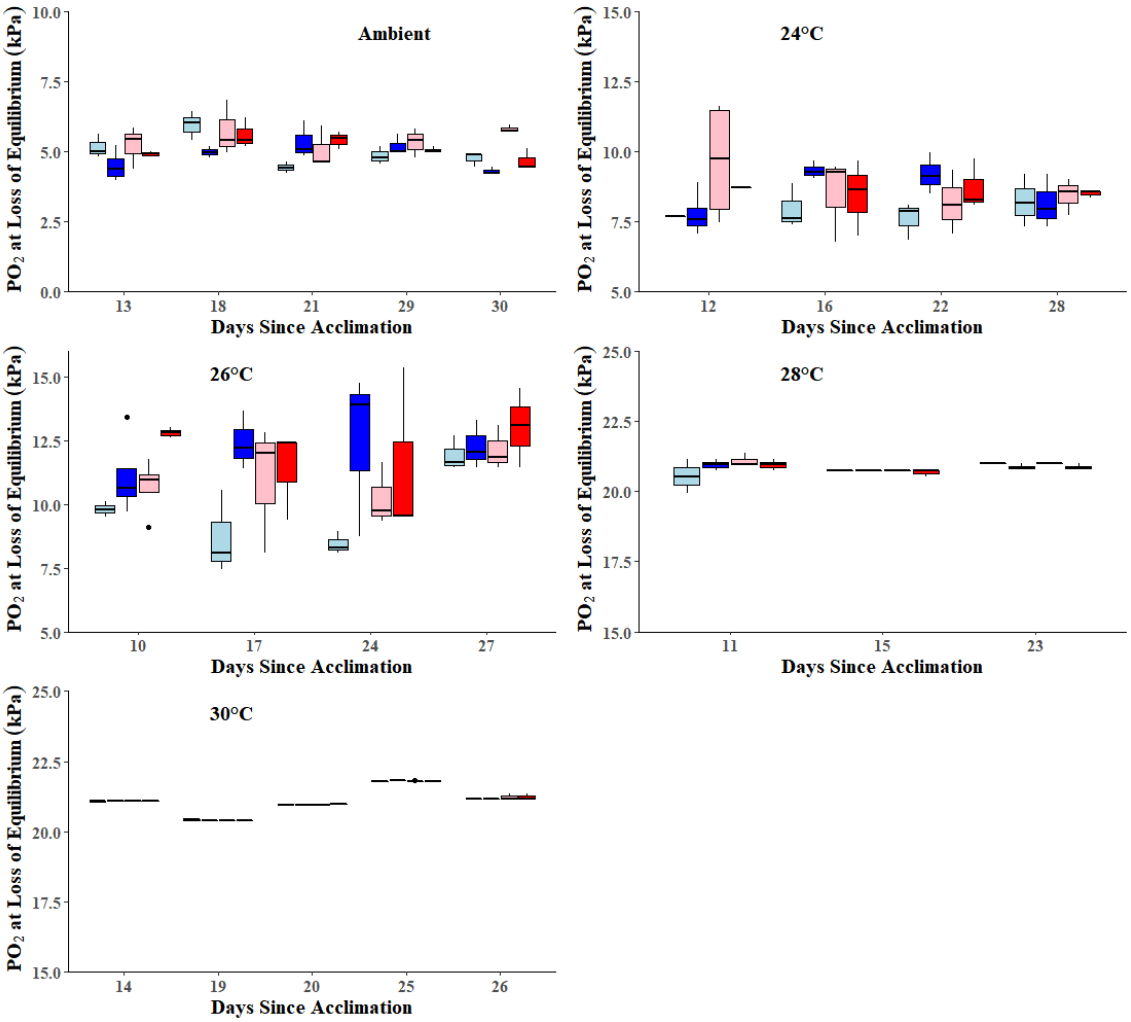

Figure 16: Oxygen tension (PO_2) at loss of equilibrium over days since end of temperature acclimation of juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures, separated by ploidy, test temperature (shown at top of panel) and previous temperature acclimation group (diploids at 15 and 18°C in light and dark blue, respectively, triploids at 15 and 18°C in pink and red, respectively; n=12 for each boxplot, with the exception of ambient boxes having n=15). Days represent time since being removed from a 4-week 18°C acclimation. Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

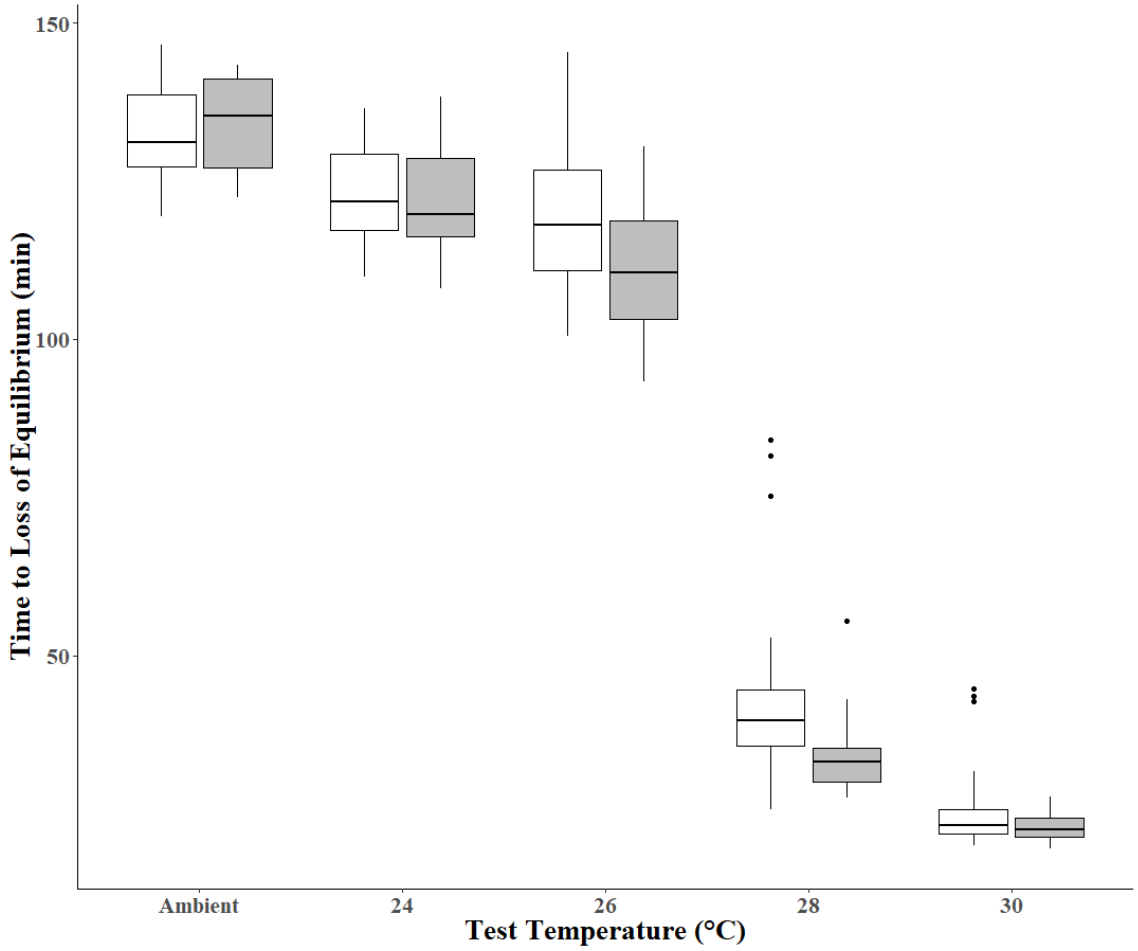

Figure 17: Time to loss of equilibrium of pooled diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures, separated by previous temperature acclimation group (15 and 18°C in white and light grey, respectively; n=24 for each boxplot, with the exception of the ambient temperature having 30 per box). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

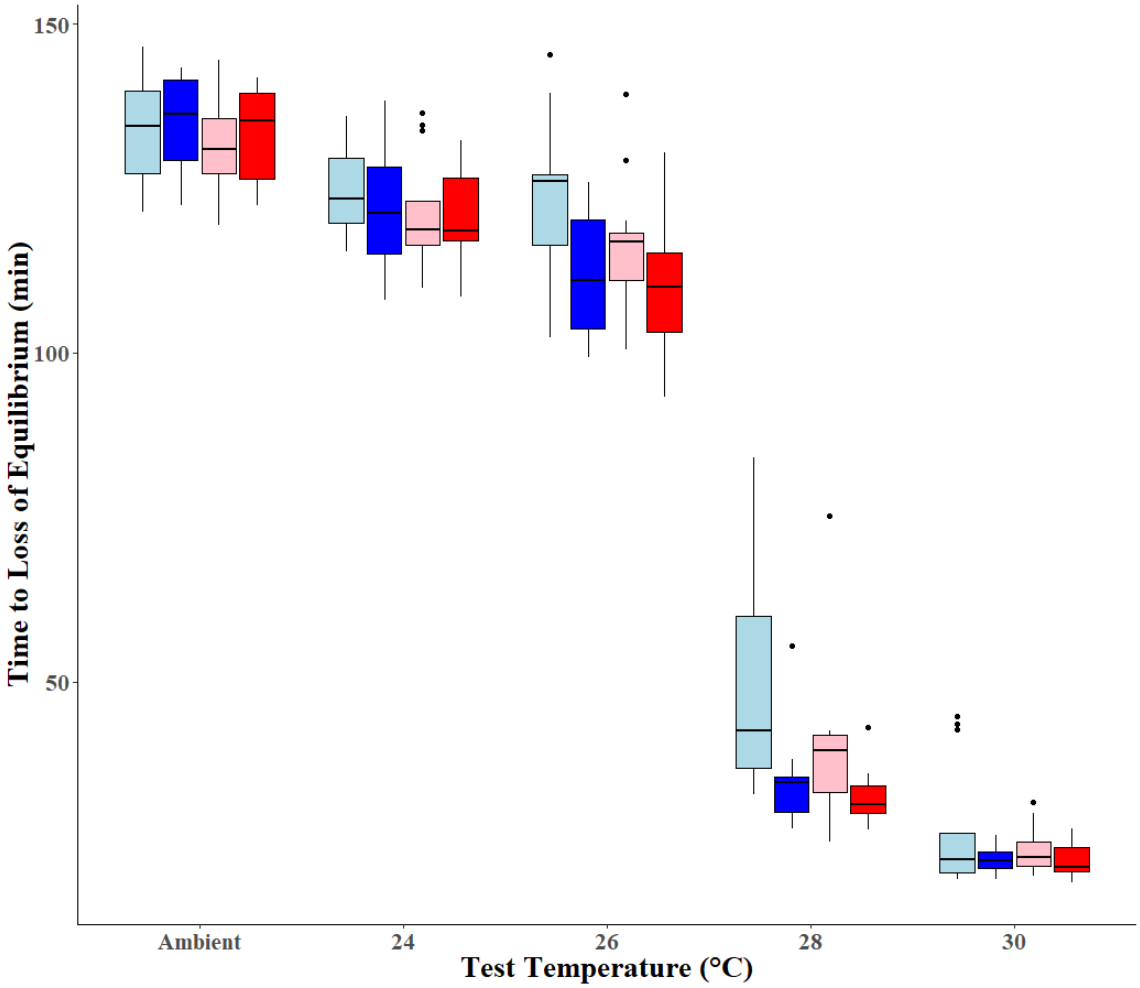

Figure 18: Time to loss of equilibrium of juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures, separated by ploidy and previous temperature acclimation group (diploids at 15 and 18°C in light and dark blue, respectively, triploids at 15 and 18°C in pink and red, respectively; n=12 for each boxplot, with the exception of ambient boxes having n=15). Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

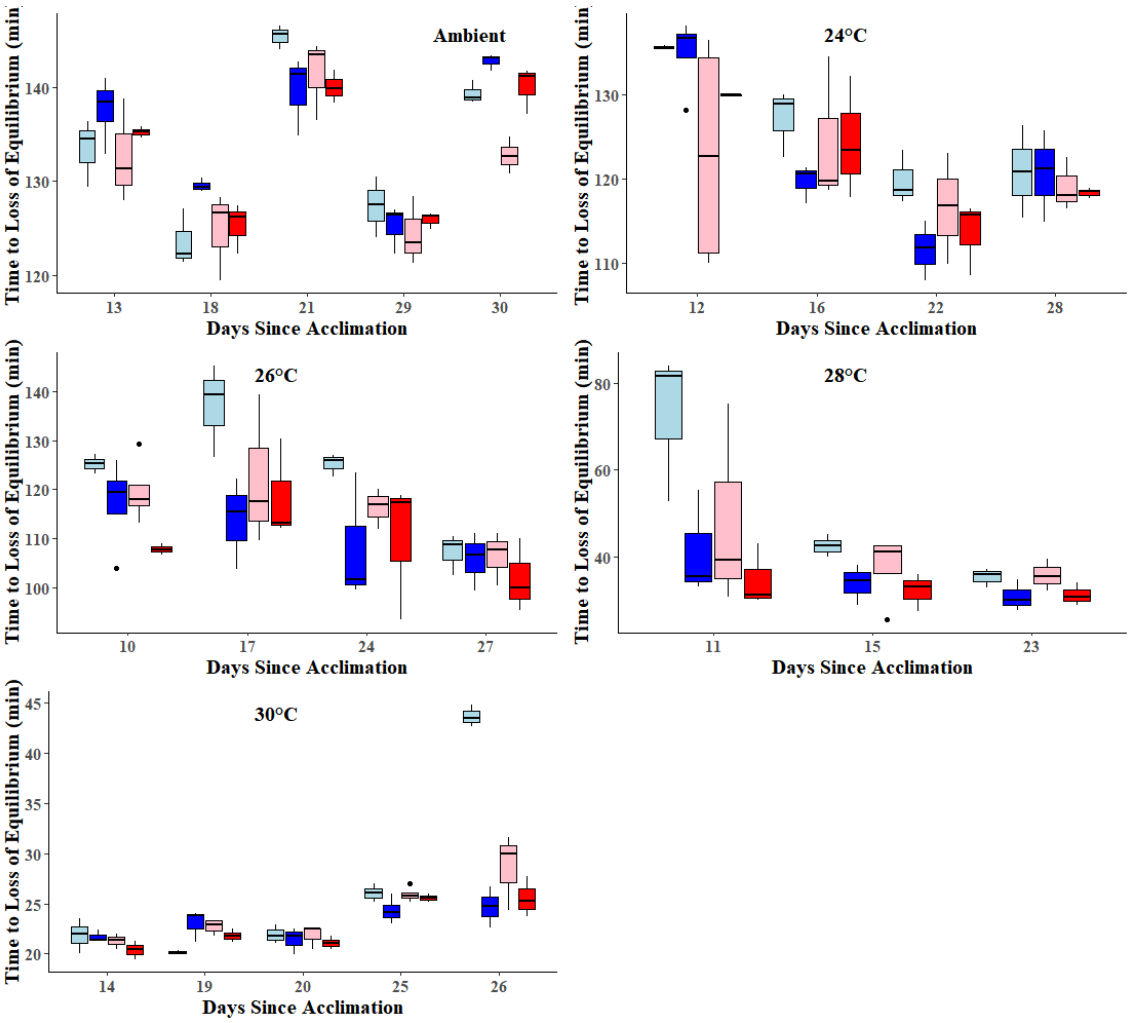

Figure 19: Time to loss of equilibrium over days since end of temperature acclimation of juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures, separated by ploidy, test temperature (shown at top of panel) and previous temperature acclimation group (diploids at 15 and 18°C in light and dark blue, respectively, triploids at 15 and18°C in pink and red, respectively; n=12 for each boxplot, with the exception of ambient boxes having n=15). Days represent time since being removed from a 4-week 18°C acclimation. Box represents the 25-75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

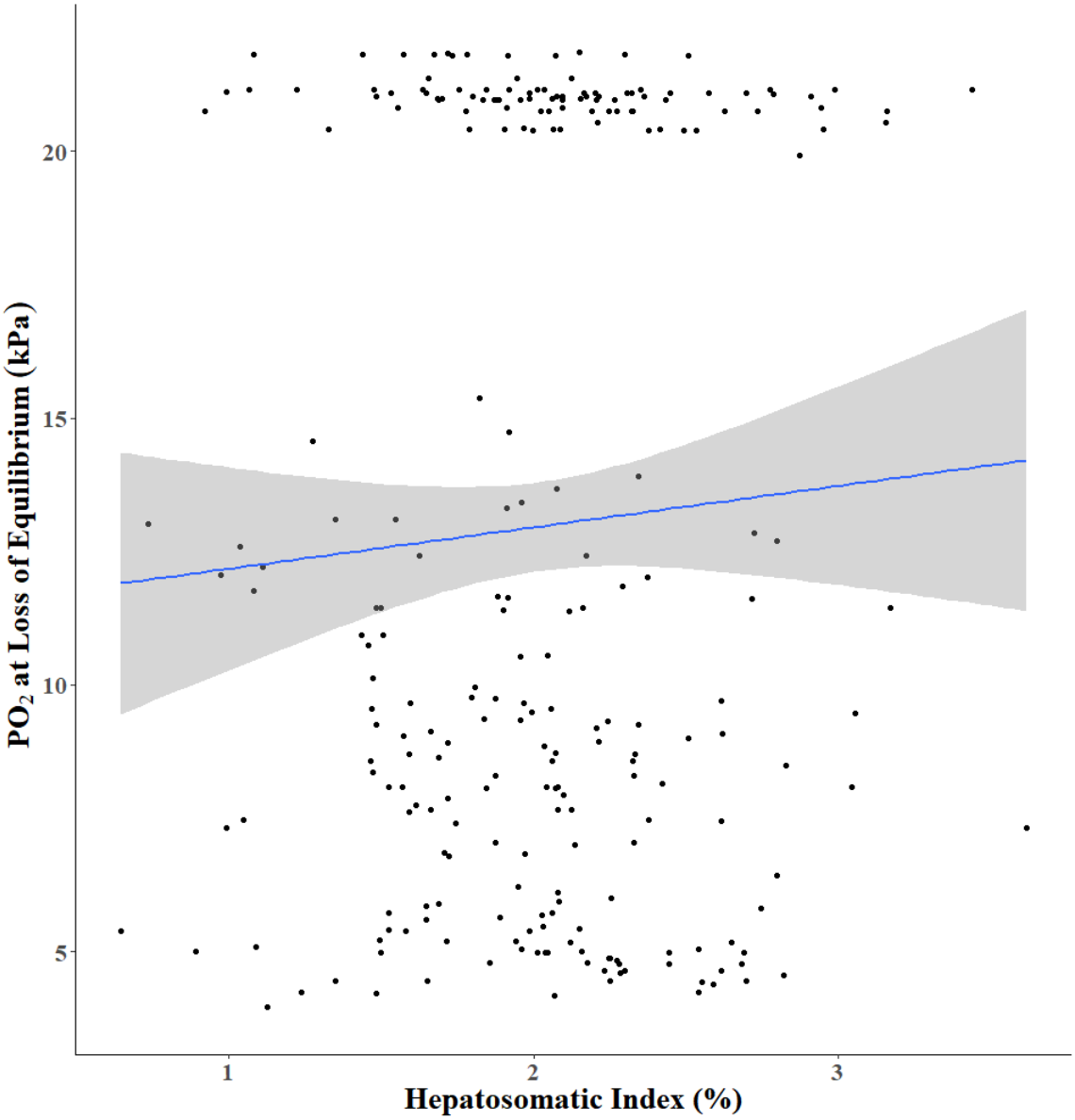

Figure 20: The effect of hepatosomatic index on PO_2 at loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures (ambient, 24, 26, 28 and 30°C; n=252). Blue line represents linear regression, grey area is 95% confidence interval.

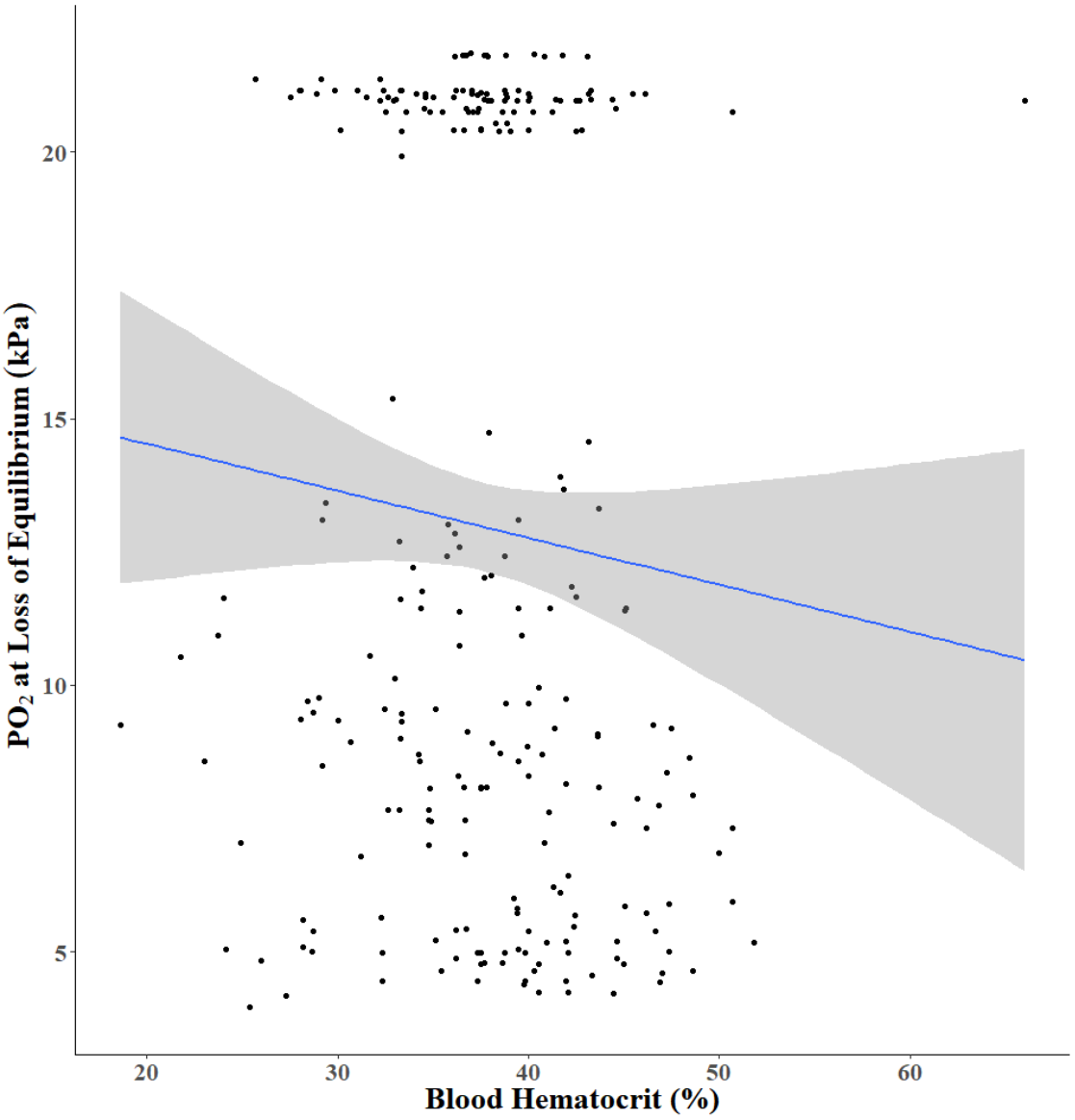

Figure 21: The effect of hematocrit on PO_2 at loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures (ambient, 24, 26, 28 and 30°C; n=252). Blue line represents linear regression, grey area is 95% confidence interval.

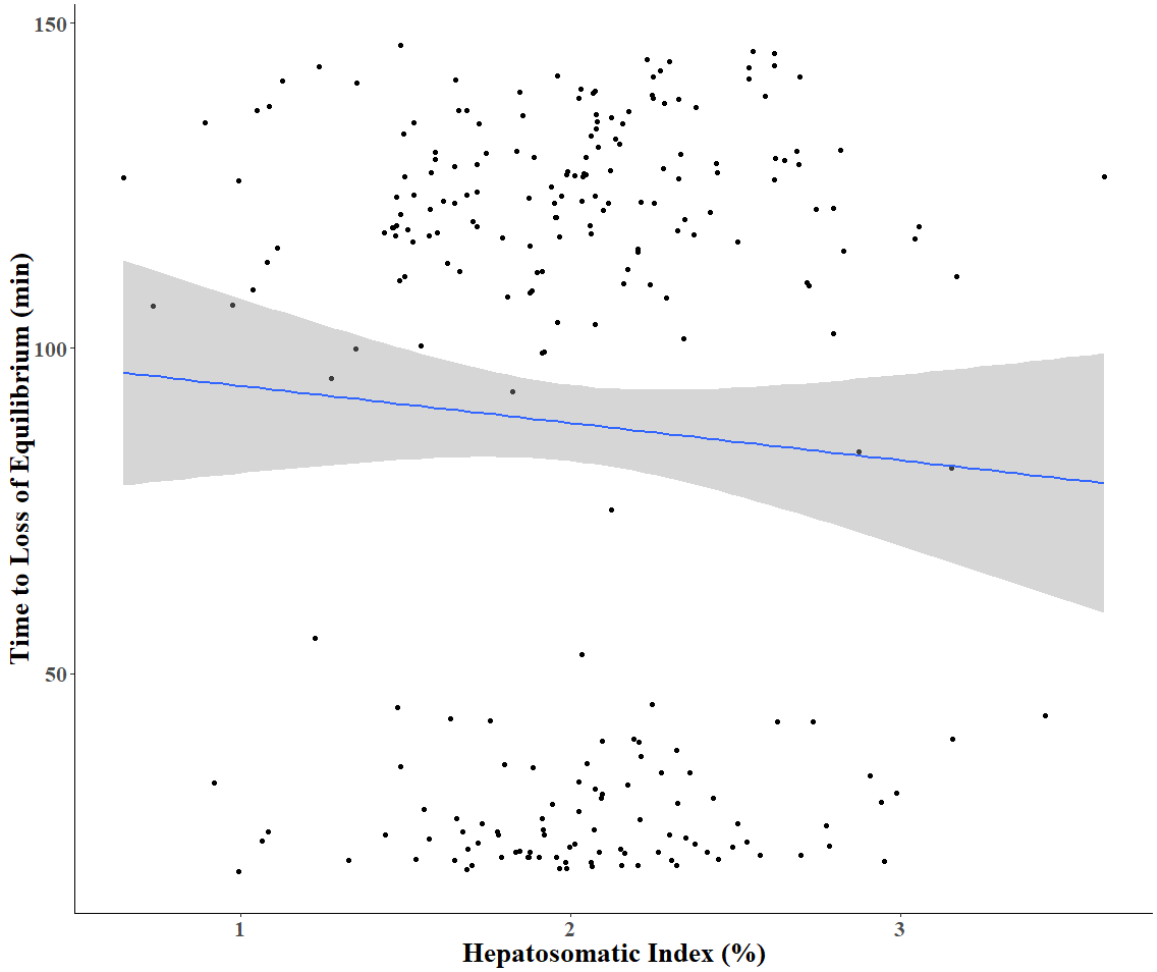

Figure 22: The effect of hepatosomatic index on time to loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures (ambient, 24, 26, 28 and 30°C; n=252). Blue line represents linear regression, grey area is 95% confidence interval.

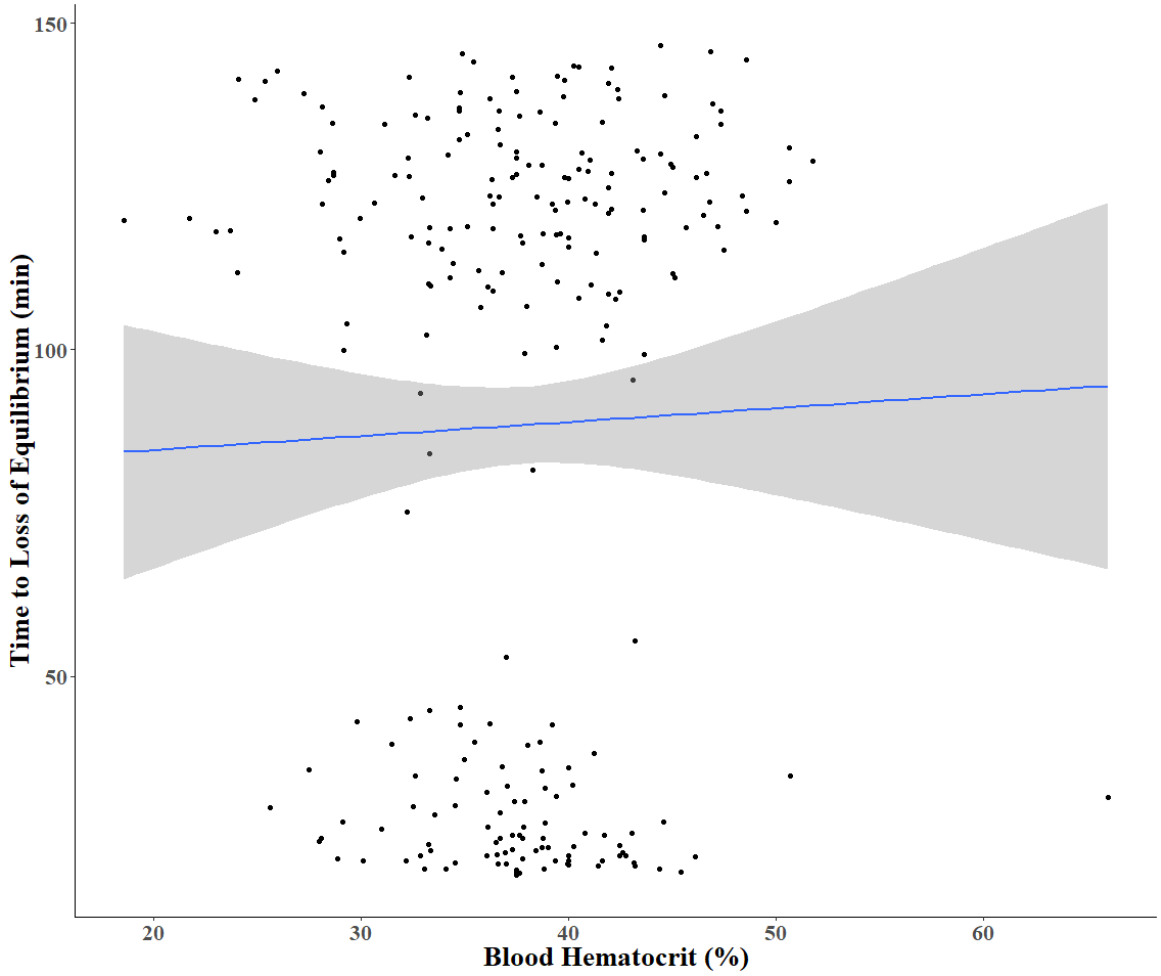

Figure 23: The effect of hematocrit on time to loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures (ambient, 24, 26, 28 and 30°C; n=252). Blue line represents linear regression, grey area is 95% confidence interval.

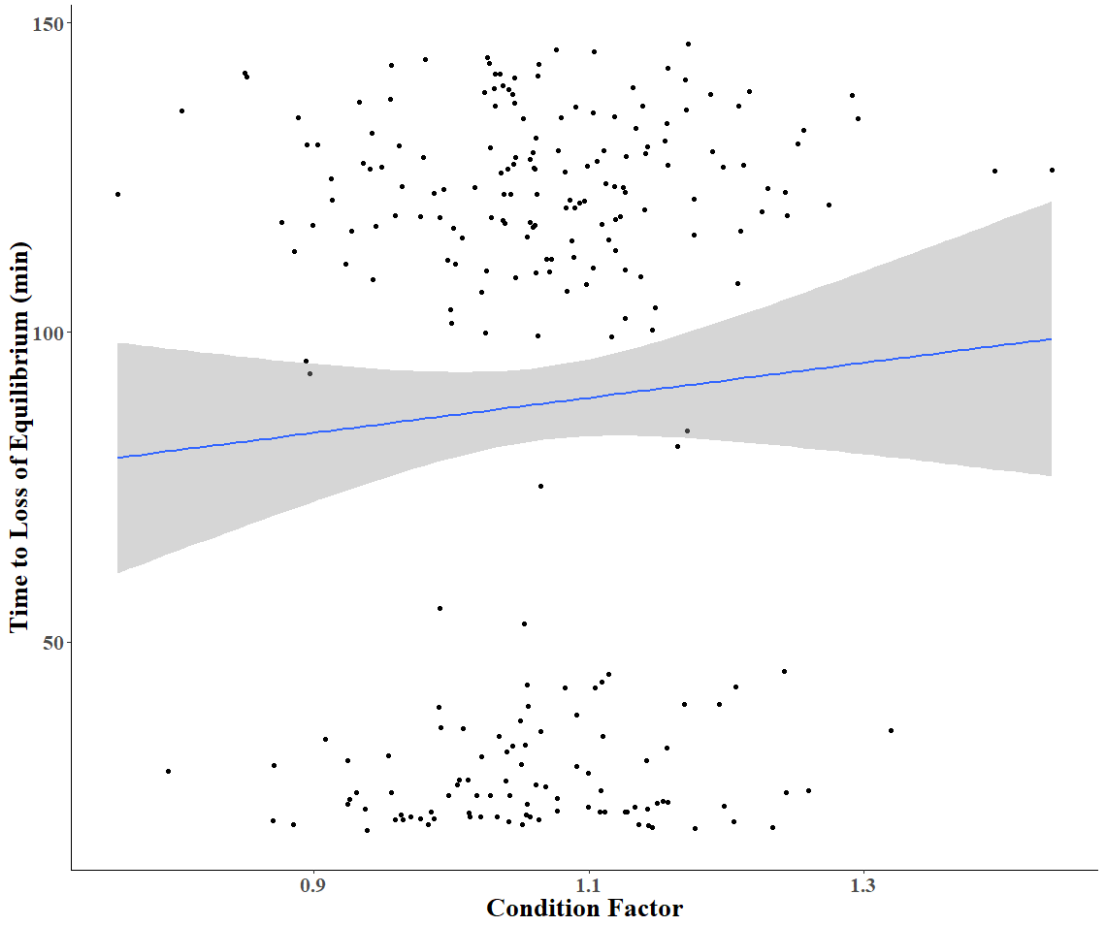

Figure 24: The effect of condition factor on time to loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to a temperature and hypoxia challenge at five different test temperatures (ambient, 24, 26, 28 and 30°C; n=252). Blue line represents linear regression, grey area is 95% confidence interval.

4. Discussion

The primary aim of this study was to examine the effects of acclimation to warm temperature and of triploidy on hypoxia tolerance of brook charr. A secondary aim was to assess the relationship between specific physiological parameters and hypoxia tolerance. The study consisted of two experiments: one designed to determine if the common salmonid response of improved hypoxia tolerance after warm temperature acclimation holds true for brook charr, and more specifically triploid brook charr and a follow-up experiment to determine if improved hypoxia tolerance would be retained after fish had been reacclimated to their original rearing temperature before being exposed to the acute hypoxia and temperature challenge. This has practical relevance because in an aquaculture setting, temperature cannot be controlled all the time, and therefore if higher rearing temperature permanently improves temperature tolerance, it could be used to improve triploid performance.

The first experiment showed that acclimating diploids and triploids to 3°C above the optimum diploid temperature (15°C: Smith and Ridgway 2019) significantly improved tolerance to hypoxia and elevated temperatures in an acute challenge with respect to both the PO_2 at loss of equilibrium and the time taken to reach loss of equilibrium. Thermal tolerance was greatly improved at higher temperatures (>26°C), and the fish did not lose equilibrium at the highest temperature tested (30°C) despite being above their presumed critical thermal maximum (Benfey *et al.,* 1997; Ellis *et al.*, 2013). This indicates a clear improvement in thermal tolerance of both diploids and triploids after only 2 weeks of acclimation to a higher temperature. Although previous studies have shown warm acclimation to improve thermal tolerance in diploids of other

salmonid species (Underwood *et al.*, 2012; Anttila *et al.*, 2014; Anttila *et al.*, 2015; Corey *et al.*, 2017), this is the first study to consider brook charr and to include triploids. This study also showed that the effect of acclimation temperature was most apparent at the higher test temperatures; at lower temperatures, the 18°C-acclimated fish did not perform much differently than the 15°C-acclimated fish.

This improved tolerance did not last after reacclimation to the original cooler temperature in the second experiment, meaning that while hypoxia tolerance is improved by warm acclimation, the effects are not long-lasting. The fish that were previously acclimated to higher temperatures actually underperformed when compared to fish that were never warm acclimated, which indicates that earlier warm acclimation that has not been continued may not be a solution for, and could even be detrimental to, performance in the long term, unless alternative methods to mitigate these negative effects are found. Days since removal from warm acclimation was significant in predicting PO_2 at loss of equilibrium and the time taken to reach loss of equilibrium in the second experiment. An initial dip in hypoxia tolerance was followed by an improvement in the following days, which could indicate hypoxia tolerance was returning to levels consistent with fish never exposed to warm acclimation, although this was not captured within the timeframe of the experiment.

Ploidy was a significant factor in determining time to loss of equilibrium in both experiments, and was either significant (first experiment) or approaching significance (second experiment) in determining PO_2 at loss of equilibrium. However, this effect of ploidy was small at any given test temperature for both experiments. This same subtle effect of ploidy on hypoxia tolerance was also found by Benfey and Devlin (2018) in

rainbow trout. Although not designed as a CT_{max} study, ploidy did not appear to influence CT_{max} based on the ability of the fish to withstand exposure to 1 hour at high temperatures in this study. Previous studies have also shown minimal ploidy effects on CT_{max} in both this and other species of salmonids (Benfey *et al.,* 1997; Ellis *et al.,* 2013; Scott *et al.,* 2015; Benfey and Devlin, 2018; Bowden *et al.,* 2018).

Reasons for reduced hypoxia tolerance in triploids have been speculated to include reduced aerobic scope (Altimiras *et al.,* 2002) or increased standard metabolic rate (O'Donnell *et al*., 2017), both of which could impact aerobic performance in situations involving stressors such as high temperature and hypoxia. The surface area to volume ratio of triploid cells is also smaller than that of diploid cells, which could cause a reduced rate of gas exchange within triploid cells, effectively slowing down cellular respiration due to a reduction in the ability to transport oxygen (Small and Benfey, 1987; Benfey, 1999; Sadler *et al.,* 2001; Leal *et al*., 2019a; 2019b). However, the difference in aerobic performance between ploidies is so subtle and variable that the overall impact on the aquaculture industry would likely be minor in comparison to the benefits triploids provide by minimizing impacts on wild populations of Atlantic salmon, as well as the financial benefit of sterility reducing preharvest maturation and energy allocation to gamete production especially if all-female populations are used (Benfey, 2016).

Triploids had a lower condition factor than diploids, as also seen in many other studies comparing diploid and triploid salmonids (e.g., Ojolick *et al.,* 1995; Fjelldal and Hansen, 2010; Peruzzi *et al.,* 2018; Sambraus *et al.,* 2018). Condition factor affected time to loss of equilibrium in both experiments, but not PO_2 at loss of equilibrium, although it was approaching significance in experiment two. The effect on time to loss of

equilibrium was very subtle and differed between experiments, with increased condition factor slightly decreasing hypoxia tolerance in the first experiment but increasing it in the second experiment. This inconsistency could be attributed to the size difference of fish between the two experiments. Since the fish used for both experiments were from the same population, and the second experiment occurred 3 months after the first, the fish were older and larger, with the average weight nearly quadrupled from 11.6 to 43.3g. Furthermore, some of the males of both ploidies and diploid females could have begun the process of sexual maturation by the second experiment, which impacts both condition factor and stress tolerance. However, the impacts of condition factor on tolerance to stressors is debated within the literature, with studies noting both improved and decreased tolerance in regard to increasing condition factor (Robb and Abrahams, 2003; Sloman *et al.*, 2006), consistent with the conflicting results of the two experiments in this study.

In the first experiment, RVM was significantly lower in warm-acclimated fish, but previously warm-acclimated fish had a higher RVM in the second experiment. While majority of salmonid studies show little change or a slight reduction in RVM in response to warm acclimation (Klaiman *et al.*, 2011; Anttila *et al*., 2015; Keen *et al.*, 2016), an increase in RVM with warm acclimation has been shown in both Arctic charr and Atlantic salmon (Gamperl *et al.*, 2020). This may indicate that different salmonid species have very specific responses to temperature acclimation and that this can vary with age, which may be due to their thermal tolerances. For example, Arctic charr are a stenothermal species that live in more Northern habitats than salmon, meaning their warm temperature tolerance is reduced (Anttila *et al*., 2015). Each species only has a certain capacity for acclimation as well, meaning for example that they can only increase

or decrease RVM to a certain extent. The RVM has been linked to performance during exposure to stressors because it is a limiting factor on the ability of the fish heart to supply oxygen-rich blood to their tissues (Farrell *et al.*, 2009). Another explanation for the difference between experiments is the differing times for acclimation between this and other studies, and how this could have differing effects on the measured parameters. The fish used for the first experiment were also very small (average weight 11.6g), and therefore had very small ventricles to remove and subsequently weigh, which could have reduced the accuracy of the measurements taken. RVM measurements are usually taken in addition to measurements of the ratio of compact to spongy myocardium, which was not possible for either of these studies but could have provided additional insights into the cardiac condition of these fish.

HSI was significant in determining hypoxia tolerance in terms of both PO_2 at loss of equilibrium and time at loss of equilibrium in both experiments. Acclimation temperature has previously been shown to impact HSI (Strobel *et al.*, 2012; Nyboer and Chapman, 2018), however, neither of these studies were performed on salmonids. A decreased HSI has also been shown to be a predictor of hypoxia tolerance, a trend that was also present in this study (De Boeck *et al.*, 2013). HSI is often used as an indicator of energy stores in fish (Chellappa *et al.*, 1995) and can be influenced by factors such as age and seasonality. A reduction in HSI is indicative of reduced energy availability which could be related to the chronic stressor of being raised in elevated temperatures (Nyboer and Chapman, 2018), as was the case of diploid brook charr acclimated to 15°C having a higher average HSI than diploids acclimated to 18°C in the first experiment. It should be noted that the opposite was true for triploids, with the fish acclimated to 18°C having a

higher average HSI. Most studies categorize triploids as having a lower HSI (e.g., Johnson *et al.,* 1986; Felip *et al.,* 2001), however, there have been studies on triploid salmonids that show them to have higher HSI (Cantas *et al*., 2011; Kizak *et al.,* 2013). The reason for the inconsistencies between ploidies and acclimation temperatures is not clear and was not present in the second experiment.

While blood hemoglobin concentration did not affect hypoxia tolerance in the first experiment, triploid fish had lower blood hemoglobin on average, which has been shown in some similar studies (Sambraus *et al.,* 2018), but other studies have found triploids to have the same or higher total blood hemoglobin concentration as diploids (Stillwell and Benfey, 1996; Cogswell *et al.,* 2002). Fish acclimated to 18°C had lower blood hemoglobin on average as well, which is contradictory to studies showing warm acclimation to increase blood hemoglobin levels in white sturgeon (*Acipenser transmontanus*) of varying ploidies (Leal *et al.,* 2018) and diploid Atlantic salmon incrementally acclimated to warm temperatures (Gamperl *et al.,* 2020). This increase is often attributed to an increase in erythropoietin production generally brought on by stressors such as hypoxia and heat acclimation (Ely *et al.,* 2014). It should be noted that sturgeon may not show responses typical of salmonids because spontaneous polyploidy is quite common in sturgeon (up to 33% of the time; Schreier *et al.,* 2013) compared to Atlantic salmon (2% in an aquaculture setting, less than 1% in wild populations; Jørgensen *et al.*, 2018). Another possible confounding factor is that blood was frozen after sampling for up to 40 days and then thawed before the hemoglobin assay occurred. Little research has been done on how freezing blood impacts the results of blood hemoglobin assays.

Hematocrit levels are often shown to be the same between diploids and triploids, as was shown in the second experiment (Small and Benfey, 1987; Cogswell *et al*., 2002). Hematocrit did not differ significantly between acclimation temperatures, consistent with other studies in triploid sturgeon (Leal *et al.*, 2019a; 2019b) but contradictory to studies on diploid Atlantic salmon that showed warm acclimation to increase hematocrit (Gamperl *et al.*, 2020). Hematocrit had a significant effect on both PO_2 at loss of equilibrium and time to loss of equilibrium, indicating that hypoxia tolerance decreases with decreasing hematocrit. This is consistent with studies on triploid sturgeon showing a decrease in hematocrit after exposure to a stressor (Leal *et al.*, 2019a). Gamperl *et al.* (2020) found that salmon acclimated to hypoxia over longer periods of time had higher hematocrit levels, however, this was over a period of weeks and not acute exposure such as was done in this study.

Two limitations of this study include the use of juveniles, which are generally hardier than adults and can therefore better tolerate stressors such as high temperature and hypoxia (Fowler *et al.*, 2009; Dupont-Prinet *et al.*, 2013), and its duration, as fish were on average larger by the end of the study, which could have impacted hypoxia tolerance. Additionally, other studies have shown species-specific responses to high temperature and hypoxia in salmonids (Anttila *et al.*, 2015), so the results of this study are merely a starting point to the larger goal of improving hypoxia tolerance in triploid fish.

4.1 Conclusion

The results from both experiments indicate that ploidy plays a part in the performance of fish in response to stressors such as hypoxia and high temperatures. While the reasons for the difference between ploidies remains unconfirmed, they may be explained largely by the difference in cell size between ploidies. Triploids generally have an increased nuclear and cell volume due to the increased amount of genetic material (Small and Benfey, 1987). Triploid fish also have fewer cells to compensate for increased cell size, such that triploids are usually the same size as diploids (Small and Benfey, 1987). This increase in cell size has an impact on the surface area to volume ratio of the cells, thereby affecting diffusion rate across the cell membrane. For instance, the reduced surface area to volume ratio of triploid cells could feasibly reduce the efficiency of oxygen uptake (Sadler *et al*., 2000), having major impact on the ability of the fish to perform aerobically. Some studies have suggested a reduced aerobic scope in triploid fish, including brook charr (O'Donnell *et al.* 2017). Triploid fish have also been shown to have difficulty using anaerobic pathways and restoring metabolites at elevated temperatures (Hyndman *et al.*, 2003), further reducing their ability to perform in hypoxic conditions.

Increased cell size in triploids may also affect heart size (and more specifically ventricle size) and the relative thickness of the ventricle's compact to spongy myocardium layers, all of which are closely linked to the ability of an organism to tolerate different stressors, including but not limited to hypoxia and high temperature (Gamperl and Farrell, 2004; Farrell *et al*., 2007; Klaiman *et al*., 2011; Keen *et al*., 2016, 2017). Similarly, changes in cell size may affect gill morphology and surface area (e.g.,

Sadler *et al.*, 2001), thereby impacting effective oxygen uptake. Traits such as these have been shown to change in response to hypoxic conditions in diploids, and the ability to tolerate hypoxia is at least partially based on functional respiratory surface area and size of the interlamellar cell mass (Sollid *et al.*, 2003; Mandic *et al.*, 2009; Nilsson *et al.*, 2012). Unfortunately, examining cardiac and gill characteristics was beyond the scope of this study, but the relevant tissues have been retained for future analysis.

The aquaculture industry continues to grow to meet global demand for food (Food and Agriculture Organization, 2018), and if this growth continues it could have a major impact on the surrounding environment. For increased sustainability, it is imperative that new technologies be adopted to minimize and mitigate negative impacts of the industry (Food and Agriculture Organization, 2018). The results of this study indicate that, with further modification, acclimating fish to slightly higher temperatures could be beneficial to the aquaculture industry in dealing with high water temperature and hypoxic events. Triploid fish acclimated to higher temperatures consistently outperformed diploids at more traditional rearing temperatures. While ploidy was statistically significant in determining hypoxia tolerance, the difference between ploidies at the same acclimation temperature was negligible, making warm temperature acclimated triploids a viable solution for the aquaculture industry to minimize impacts of farmed fish mating with wild fish (Benfey, 2016). This lends itself to the broader notion that while triploids may be the same species as diploids, subtleties brought about by the differences between ploidies could have an impact on their effectiveness as an aquaculture species. Other studies on different aspects of rearing have already shown improved triploid performance with changes in conditions such as diet and indicate that dietary changes can reduce

occurrence of deformities such as cataracts (Taylor *et al.*, 2015; Peruzzi *et al.*, 2018). This means that triploid-specific husbandry protocols are necessary, and this research in combination with other research on triploid temperature and hypoxia tolerance as well as other aspects of husbandry could be useful in determining their optimal rearing conditions for the aquaculture industry.

4.2 Future Studies

Both of these studies could also be repeated using Atlantic salmon instead of brook charr. While brook charr are a financially and culturally important species in their own right, this study aimed to use them as a model for Atlantic salmon, a widely used species in aquaculture. Temperature has been shown to affect smoltification (McCormick *et al.*, 1996; Vargas-Chacoff *et al.*, 2018), and rearing temperatures designed to optimize a multitude of important factors such as growth, smoltification, and performance in high temperature and hypoxia could be assessed in Atlantic salmon. Apart from smoltification, growth and performance could also be assessed long-term in response to high temperature rearing and hypoxia in brook charr.

The first experiment showed a significant improvement in thermal tolerance after only two weeks of acclimation. Other studies have also theorized that thermal tolerance is determined at an embryonic stage in development (e.g., Scott and Johnston, 2012) so it would be interesting to conduct a long-term experiment in which fish of both ploidies are acclimated to warm temperatures from shortly after fertilization to see if this effect is greater, and if it would be worthwhile to raise fish at higher temperatures from the very beginning. It would also be interesting to conduct this experiment over the lifetime of a

cohort of fish, sampling every few months to see the different impacts on different stages of development and to determine if these effects remain consistent over time.

The second experiment showed an immediate reduction in tolerance following acclimation, but over time showed the beginning of an improvement in tolerance, which may indicate that if the study had continued for longer, there may have been further improvement to the hypoxia tolerance of the previously warm-acclimated fish. Another interesting take would be to repeat this experiment, but with fish acclimated to a warmer temperature since embryonic stages and then subsequently removed from warm acclimation for a period of time, as with the suggestion for the first experiment, to see if longer acclimation from earlier stages in development could in fact improve the performance in high temperature and hypoxia for longer periods of time.

Both experiments could also be repeated at different acclimation temperatures to determine if adding 3°C to their current temperature maximizes hypoxia tolerance, or if different temperatures have better effects. Acclimating the fish to different hypoxic conditions, a combination of hypoxia and warm temperatures, and cycling hypoxia and temperature are just a few of the many studies that could be performed to expand on this work and optimize triploid performance for aquaculture and globally changing temperatures.

5. References

Altimiras, J., Axelsoon, M., Claireaux, G., Lefrancois, C., Mercier, C., and Farrell, A.P. 2002. Cardiorespiratory status of triploid brown trout during swimming at two acclimation temperatures. *Journal of Fish Biology* 60: 102-116.

Anttila, K., Couturier, C.S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G.E., and Farrell, A.P. 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nature Communications* 5: 4252.

Anttila, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppänen, E., Kolari, I., and Nikinmaa, M. 2015. Warm acclimation and oxygen depletion induce species-specific responses in salmonids. *Journal of Experimental Biology* 218: 1471-1477.

Atkins M.E. and Benfey T.J. 2008. Effect of acclimation temperature on routine metabolic rate in triploid salmonids. *Comparative Biochemistry and Physiology, Part A* 149: 157-161.

Benfey, T.J. 1999. The physiology and behaviour of triploid fishes. *Reviews in Fisheries Science* 7: 39-67.

Benfey, T.J. 2016. Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. *Reviews in Aquaculture* 8: 264-282.

Benfey, T.J. and Devlin, R.H. 2018. Ploidy has minimal effect on hypoxia tolerance at high temperatures in rainbow trout (*Oncorhynchus mykiss*). *Physiological and Biochemical Zoology* 6: 1091-1101.

Benfey, T.J., McCabe, L., and Pepin, P. 1997. Critical thermal maxima of diploid and triploid brook charr, *Salvelinus fontinalis. Environmental Biology of Fishes* 49: 259-264.

Benfey, T.J. and Sutterlin, A.M. 1984. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). *Aquaculture* 36: 359-367.

Benfey, T.J., Sutterlin, A.M. and Thompson, R.J. 1984. Use of erythrocyte measurements to identify triploid salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 41: 980-984.

Bowden, A.J., Andrewartha, S.J., Elliot, N.G., Frappell, P.B., and Clark, T.D. 2018. Negligible differences in metabolism and thermal tolerance between diploid and triploid Atlantic salmon (*Salmo salar*). *Journal of Experimental Biology* 221: jeb166975.

Breau, C., Cunjak, R.A., and Bremset, G. 2007. Age-specific aggregation of wild juvenile Atlantic salmon *Salmo salar* at cool water sources during high temperature events. *Journal of Fish Biology* 71: 1179-1191.

Breau, C., Cunjak, R.A., and Peake, S.J. 2011. Behaviour during elevated water temperatures: can physiology explain movement of juvenile Atlantic salmon to cool water? *Journal of Animal Ecology* 80: 844-853.

Cantas, L., Fraser, T.W.K., Fjelldal, P.G., Mayer, I., and Sørum, H. 2011. The culturable intestinal microbiota of triploid and diploid juvenile Atlantic salmon (*Salmo salar*) - a comparison of composition and drug resistance. *BMC Veterinary Research* 7: 71.

Chellappa, S., Huntingford, F.A., Strang, R.H.C., and Thomson, R.Y. 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *Journal of Fish Biology* 47: 775-787.

Chittenden, C.M., Rikardsen, A.H., Skilbrei, O.T., Davidsen, J.G., Haltunen, E., Skarðhamar, J., and McKinley, R.S. 2011. An effective method for the recapture of escaped farmed salmon. *Aquaculture Environment Interactions* 1: 215-224.

Cogswell, A.T., Benfey, T.J., and Sutterlin, A.M. 2002. The hematology of diploid and triploid transgenic Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* 24: 271-277.

Cook, B. and McGaw, R. 1991. Management of the New Brunswick commercial salmon fishery. *Marine Policy* 15: 33-38.

Corey, E., Linnansaari, T., Cunjak, R.A., and Currie, S. 2017. Physiological effects of environmentally relevant, multi-day thermal stress on wild Atlantic salmon (*Salmo salar*). *Conservation Physiology* 5: cox014.

Committee on the Status of Endangered Wildlife in Canada. 2010. COSEWIC assessment and status report on the Atlantic Salmon *Salmo salar* (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population, Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy population) in Canada. xlvii+136 (Ottawa, 2010).

De Boeck, G., Wood, C.M., Iftikar, F., Matey, V., Scott, G.R., Sloman, K.A., de Nazaré Paula da Silva, M., Almeida-Vail, V.M.F., and Val, A.L. 2013. Interactions between hypoxia tolerance and food deprivation in Amazonian Oscars, *Astronotus ocellatus. Journal of Experimental Biology* 216: 4590-4600.

Dejours, P. 1975. Principles of Comparative Respiratory Physiology. North-Holland, Amsterdam.

Dempster, T., Wright, D. and Oppedal, F. 2016. Identifying the nature, extent and duration of critical production periods for Atlantic salmon in Macquarie Harbour, Tasmania, during summer. Fisheries Research & Development Corporation Project No: 2016-229 (Fisheries Research and Development Corporation, Canberra).

Dunfield, R.W. 1985. The Atlantic salmon in the history of North America. *Canadian Special Publication of Fisheries and Aquatic Sciences* 80: 181p.

Dupont-Prinet, A., Vagner, M., Chabot, D. and Audet, C. 2013. Impact of hypoxia on the metabolism of Greenland halibut (*Reinhardtius hippoglossoides*). *Canadian Journal of Fisheries and Aquatic Sciences* 70: 461-469.

Ellis, L.E., Sacobie, C.F.D., Kieffer, J.D., and Benfey, T.J. 2013. The effects of dissolved oxygen and triploidy on critical thermal maximum in brook charr (*Salvelinus fontinalis*). *Comparative Biochemistry and Physiology, Part A* 166: 426-433.

Ely, B.R., Lovering, A.T., Horowitz, M., and Minson, C.T. 2014. Heat acclimation and cross tolerance to hypoxia: bridging the gap between cellular and systemic responses. *Temperature* 1:107-114.

Evans, D.H., Piermarini, P.M., and Choe, K.P. 2005. The multifunctional fish gill: osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85: 97-177.

Farrell, A.P., Eliason, E.J., Sandblom, E., and Clark, T.D. 2009. Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835-851.

Farrell, A.P., Simonot, D.L., Seymour, R.S., and Clark, T.D. 2007. A novel technique for estimating the compact myocardium in fishes reveals surprising results for an athletic air-breathing fish, the Pacific tarpon. *Journal of Fish Biology* 71: 389-398.

Felip, A., Piferrer, F., Zanuy, S., and Carrillo, M. 2001. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. *Journal of Fish Biology* 58: 76-88.

Ferguson, A., Fleming, I., Hindar, K., Skaala, Ø., McGinnity, P., Cross, T.F. and Prodöhl, P. 2007. *Farm Escapes*. In: The Atlantic salmon: Genetics, Conservation and Management (Verspoor, E., Stradmeyer, L. and Nielsen, J. L., eds.). Blackwell Publishing Ltd, pp. 357-398.

Fjelldal, P.G. and Hansen, T. 2010. Vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture* 309: 131-136.

Food and Agriculture Organization. 2018. *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals*. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Fowler, S.L., Hamilton, D., and Currie, S. 2009. A comparison of the heat shock response in juvenile and adult rainbow trout (*Oncorhynchus mykiss*) – implications for increased thermal sensitivity with age. *Canadian Journal of Fisheries and Aquatic Sciences* 66: 91-100.

Gamperl, A.K., Ajiboye, O.O., Zanuzzo, F.S., Sandrelli, R.M., Peroni, E.F.C., and 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.

Gamperl, A.K. and Farrell, A.P. 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. *The Journal of Experimental Biology* 207: 2539-2550.

Glover, K.A., Pertoldi, C., Besnier, F., Wennevik, V., Kent, M., and Skaala, Ø. 2013. Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. *BMC Genetics* 14: 74.

Glover, K.A., Solberg, M.F., Besnier, F., and Skaala, Ø. 2018. Cryptic introgression: evidence that selection and plasticity mask the full phenotypic potential of domesticated Atlantic salmon in the wild. *Scientific Reports* 8: 13966.

Government of New Brunswick. 2019. 2018 Aquaculture Sector Review. Retrieved from:https://www2.gnb.ca/content/dam/gnb/Departments/10/pdf/Publications/Aqu/ReviewAquaculture2017.pdf

Gräns, A., Jutfelt, F., Sandblom, E., Jönssen, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I., Björnsson, B.T., Sundell, K., and Axelsson, M. 2014. Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. *Journal of Experimental Biology* 217: 711-717.

Hansen, T.J., Olsen, R.E., Stien, L., Oppedal, F., Torgersen, T., Breck, O., Remmen, M., Vagseth, T., Fjelldal, P.G. 2015. Effect of water oxygen level on performance of diploid and triploid Atlantic salmon post-smolts reared at high temperatures. *Aquaculture* 435: 354-360.

Heino, M., Svåsand, T., Wennevik, V, and Glover, K.A. 2015. Genetic introgression of farmed salmon in native populations quantifying the relative influence of population size and frequency of escapees. *Aquaculture and Environment Interactions* 6: 185-190.

Hyndman, C.A., Kieffer, J.D., Benfey, T.J. 2003. Physiology and survival of triploid brook trout following exhaustive exercise in warm water. *Aquaculture* 221: 629–643.

Intergovernmental Panel on Climate Change. 2013. *Climate change 2013. The physical science basis*. Working group I contribution to the fifth assessment report (eds TF Stocker, D Qin, G-K Plattner, M Tignor, SK Allen, J Boschung, A Nauels, Y Xia, V Bex, PM Midgley), pp. 255–317 New York, NY: Cambridge University Press.

Jensen, A.J., Karlsson, S., Fiske, P., Hansen, L.P., Hindar, K., and Østborg, G.M. 2013 Escaped farmed Atlantic salmon grow, migrate and disperse throughout the Arctic Ocean like wild salmon. *Aquaculture Environment Interactions* 3: 223–229.

Jensen, Ø., Dempster, T., Thorstad, E.B., Uglem, I., and Fredheim, A. 2010. Escapes of Fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. *Aquaculture Environment Interactions* 1: 71-83.

Jobling, M., Arnesen, A.-M., Benfey, T.J., Carter, C., Hardy, R., Le François, N., O'Keefe, R., Koskela, J. and Lamarre, S. 2010. *The Salmonids*. In: Le François, N., Jobling, M., Carter, C. and Blier, P. (Eds.) Finfish Aquaculture. ISBN: 9781845934941 CABI Publishing. pp. 234-289.

Johnson, O.W., Dickhoff, W.W., and Utter, F.M. 1986. Comparative growth and development of diploid and triploid coho salmon, *Oncorhynchus kisutch. Aquaculture* 57: 329-336.

Jørgensen, K.M., Wennevik, V., Eide Sørvik, A.G., Unneland, L., Prusov, S., Ayllon, F., and Glover, K.A. 2018. Investigating the frequency of triploid Atlantic salmon in wild Norwegian and Russian populations. *BMC Genetics* 19: 90.

Keen, A.N., Fenna, A.J., McConnell, J.C., Sherratt, M.J., Gardner, P., and Shiels, H.A. 2016. The dynamic nature of the hypertrophic and fibrotic remodeling of the fish ventricle. *Frontiers in Physiology* 6: 427.

Keen, A.N., Klaiman, J.M., Shiels, H.A., and Gillis, T.E. 2017. Temperature-induced cardiac remodelling in fish. *Journal of Experimental Biology* 220: 147-160.

Kelly, N.I., Burness, G., McDermid, J.L., and Wilson, C.C. 2014. Ice age fish in a warming world: minimal variation in thermal acclimation capacity among lake trout (*Salvelinus namaycush*) populations. *Conservation Physiology* 2: 1-14.

Kizak, V., Güner, Y., Türel, M., and Kayim, M. 2013. Comparison of growth performance, gonadal structure and erythrocyte size in triploid and diploid brown trout (*Salmo trutta fario* L, 1758). *Turkish Journal of Fisheries and Aquatic Sciences* 13: 571-580.

Klaiman, J.M., Fenna, A.J., Shiels, H.A., Macri, J., and Gillis, T.E. 2011. Cardiac remodeling in fish: strategies to maintain heart function during temperature change. *PLoS ONE* 6: e24464.

Lai, J.C., Kakuta, I., Mok, H.O.L., Rummer, J.L., and Randall, D. 2006. Effects of moderate and substantial hypoxia on erythropoietin levels in rainbow trout kidney and spleen. *Journal of Experimental Biology* 209: 2734-2738.

Leal, M.J., Clark, B.E., Van Eenennaam, J.P., Schreier, A.D., and Todgham, A.E. 2018. The effects of warm temperature acclimation on constitutive stress, immunity, and metabolism in white sturgeon (*Acipenser transmontanus*) of different ploidies. *Comparative Biochemistry and Physiology, Part A* 224: 23-34.

Leal, M.J., Van Eenennaam, J.P., Schreier, A.D., and Todgham, A.E. 2019a. Triploidy in white sturgeon (*Acipenser transmontanus*): Effects of acute stress and warm acclimation on physiological performance. *Comparative Biochemistry and Physiology, Part A* 229: 10-17.

Leal, M.J., Van Eenennaam, J.P., Schreier, A.D., and Todgham, A.E. 2019b. Diploid and triploid white sturgeon (*Acipenser transmontanus*) differ in magnitude but not kinetics of physiological responses to exhaustive exercise at ambient and elevated temperatures. *Canadian Journal of Fisheries and Aquatic Sciences* 77: 666-673.

Li, Y.-J., Gao, Y.-C., Zhou, H., Ma, H.-Y., Lin, Z.-Q., Ma, T.-Y., Sul, Y., and Arai, K. 2016. Aneuploid progenies of triploid hybrids between diploid and tetraploid loach *Misgurnus anguillicaudatus* in China. *Genetica* 144:601-609.

Lijalad, M. and Powell, M.D. 2009. Effects of lower jaw deformity on swimming performance and recovery from exhaustive exercise in triploid and diploid Atlantic salmon *Salmo salar* L. *Aquaculture* 290: 145-154.

Lucas, J.S. 2012. *Introduction.* In: Lucas, J.S. and Southgate, P.C. (eds.). Aquaculture: Farming Aquatic Animals and Plants. Blackwell Publishing, Oxford, England. pp. 295-320.

MacNutt, M.J., Hinch, S. G., Farrell, A.P., and Topp, S. 2004. The effect of temperature and acclimation period on repeat swimming performance in cutthroat trout. *Journal of Fish Biology* 65: 342-353.

Madsen, L., Arnbjerg, J., and Dalsgaard, I. 2000. Spinal deformities in triploid all-female rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* 20: 206-208.

Mandic, M., Todgham, A.E., and Richards, J.G. 2009. Mechanisms and evolution of hypoxia tolerance in fish. *Proceedings of the Royal Society B: Biological Sciences* 276: 735-744.

McBryan, T.L., Healy, T.M., Haakons, K.L., and Schulte, P.M. 2016. Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *Journal of Experimental Biology* 219: 474-484.

McCormick, J.H., Hokanson, K.E., and Jones, B.R. 1972. Effects of temperature on growth and survival of young brook trout, *Salvelinus fontinalis*. *Journal of the Fisheries Research Board of Canada* 29: 1107-1112.

McCormick, S.D., Shrimpton, J.M. and Zydlewski, J.D. 1996. Temperature effects on osmoregulatory physiology of juvenile anadromous fish. In Global Warming: Implications for Freshwater and Marine Fish (ed. C. M. Wood and D. G. Mcdonald), pp. 279-301. Cambridge: Cambridge University Press.

McDermid, J.L., Wilson, C.C., Sloan, W.N., Shuter, B.J. 2013. Intraspecific differences in thermal biology among inland lake trout populations. *Transactions of the American Fisheries Society* 142: 756–766.

McGeachy, S.A., Benfey, T.J., and Friars, G.W. 1995. Freshwater performance of triploid Atlantic salmon (*Salmo salar*) in New Brunswick aquaculture. *Aquaculture* 137: 333–341.

McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., Maoilédigh, N., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J., and Cross, T. 2003. Fitness reduction and potential extinction of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society of London B* 270: 2443-2450.

McGinnity, P., Stone, C., Taggart, J.B., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., and Ferguson, A. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES Journal of Marine Science* 54: 998-1008.

McKenzie, D.J., Shingles, A., and Taylor, E.W. 2003. Sub-lethal plasma ammonia accumulation and the exercise performance of salmonids. *Comparative Biochemistry and Physiology, Part A* 135: 515-526.

Nilsson, G.E., Dymowska, A., and Stecyk, J.A.W. 2012. New insights into the plasticity of gill structure. *Respiratory Physiology & Neurobiology* 184: 214-222.

Nyboer, E.A. and Chapman, L.J. 2018. Cardiac plasticity influenced aerobic performance and thermal tolerance in a tropical, freshwater fish at elevated temperatures. *Journal of Experimental Biology.* 221: jeb178087.

O'Donnell, K.M., MacRae, K.L., Verhille, C.E., Sacobie, C.F.D., and Benfey, T.J. 2017. Standard metabolic rate of juvenile triploid brook charr, *Salvelinus fontinalis*. *Aquaculture* 479:85-90.

O'Flynn, F., McGeachy, S.A., Friars, G.W., Benfey, T.J., and Bailey, J. K. 1997 Comparisons of cultured triploid and diploid Atlantic salmon (*Salmo salar* L.). *ICES Journal of Marine Science* 54: 1160–1165.

Ojolick, E.J., Cusack, R., Benfey, T.J., and Kerr, S.R. 1995. Survival and growth of all-female diploid and triploid rainbow trout (*Oncorhynchus mykiss*) reared at chronic high temperature. *Aquaculture* 131: 177-187.

Peruzzi, S., Hagen, Ø., and Jobling, M. 2014. Gut morphology of diploid and triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture International* 4: 1105-1108.

Peruzzi, S., Puvanendran, V., Riesen, G., Ripman Seim, R., Hagen, Ø., Martinez-Llorens, S., Falk-Petersen, I.-B., Fernandes, J.M.O., and Jobling, M. 2018. Growth and development of skeletal anomalies in diploid and triploid Atlantic salmon (*Salmo salar*) fed phosphorous-rich diets with fish meal and hydrolyzed fish protein. *PLoS ONE* 13: e0194340.

Piferrer, F., Beaumont, A., Falguiere, J.C., Flajšhans, M., Haffray, P, and Colombo, L. 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* 293: 125-156.

Pörtner, H.-O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* 88: 137-146.

Pörtner, H.-O. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology, Part A* 132: 739-761.

Pörtner, H.-O. 2010. Oxygen-and-capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology* 213: 881–893.

Pörtner, H.-O., Bock, C., and Mark, F.C. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *Journal of Experimental Biology* 220: 2685-2696.

Purser, J. and Forteath, N. 2012. *Salmonids*. In: Lucas, J.S. and Southgate, P.C. (eds.), Aquaculture: Farming Aquatic Animals and Plants. Blackwell Publishing, Oxford, England. pp. 295-320.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Robb, T. and Abrahams, M.V. 2003. Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? *Journal of Fish Biology* 62: 1067-1081.

Sadler, J., Wells, R.M.G., Pankhurst, P.M. and Pankhurst, N.W. 2000. Blood oxygen transport, rheology and haematological responses to confinement stress in diploid and triploid Atlantic salmon, *Salmo salar*. *Aquaculture* 184: 349-361.

Sadler, J., Pankhurst, P.M., and King, H.R. 2001. High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar*). *Aquaculture* 198: 369-386.

Sambraus, F., Remen, M., Olsen, R., Hansen, T., Waagbø, R., Torgersen, T., Lock, E.J., Imsland, A., Fraser, T.W.K., Fjelldal, P. 2018. Changes in water temperature and oxygen: the effect of triploidy on performance and metabolism in large farmed Atlantic salmon. *Aquaculture Environment Interactions* 10: 157-172.

Sandblom, E., Gräns, A., Axelsson, M., and Seth, H. 2014. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. *Proceedings of the Royal Society of Publishing B* 281: 20141490.

Schreier, A.D., May, B., and Gille, D.A. 2013. Incidence of spontaneous autopolyploidy in cultured populations of white sturgeon, *Acipenser transmontanus*. *Aquaculture* 416-417:141-145.

Scott, G.R. and Johnston, I.A. 2012. Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *PNAS* 109: 14247-14252.

Scott, M.A., Dhillon, R.S., Schulte, P.M., and Richards, J.G. 2015. Physiology and performance of wild and domestic strains of diploid and triploid rainbow trout (*Oncorhynchus mykiss*) in response to environmental challenges. *Canadian Journal of Fisheries and Aquatic Sciences* 72: 125-134.

Secor, D.H. and Gunderson, T.E. 1998. Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon, *Acipenser oxyrinchus. Fishery Bulletin* 96: 603-613.

Skilbrei, O.T. and Jørgensen, T. 2010. Recapture of cultured salmon following a large-scale escape experiment. *Aquaculture Environment Interactions* 1: 107-115.

Sloman, K.A., Wood, C.M., Scott, G.R., Wood, S., Kajimura, M., Johannsson, O.E., Almeida-Val, V.M.F., Val, A.L. 2006. Tribute to R.G. Boutilier: The effect of size on the physiological and behavioural responses of oscar, *Astronotus ocellatus,* to hypoxia. *Journal of Experimental Biology* 209: 1197–1205.

Small, S. and Benfey, T.J. 1987. Cell size in triploid salmon. *Journal of Experimental Zoology* 241: 339-342.

Smith, D.A. and Ridgway, M.S. 2019. Temperature selection in Brook Charr: lab experiments, field studies, and matching the Fry curve. *Hydrobiologia* 840: 143-156.

Sollid, J., De Angelis, P., Gundersen, K., and Nilsson, G.E. 2003. Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *Journal of Experimental Biology* 206: 3667-3673.

Solstorm, D., Oldham, T., Solstorm, F., Klebert, P., Stien, L.H., Vågseth, T., Oppedal, F. 2018. Dissolved oxygen variability in a commercial sea-cage exposes farmed Atlantic salmon to growth limiting conditions. *Aquaculture* 486: 122–129.

Stehfest, K.M., Carter, C.G., McAllister, J.D., Ross, J.D., and Semmens, J.M. 2017. Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors. *Scientific Reports* 7: 4545.

Stillwell, E.J. and Benfey, T.J. 1996. Hemoglobin level, metabolic rate, opercular abduction rate and swimming efficiency in female triploid brook trout (*Salvelinus fontinalis*). *Fish Physiology and Biochemistry* 15: 377-383.

Stitt, B.C., Burness, G., Burgomaster, K.A., Currie, S., McDermid, J.L., and Wilson, C.C. 2013. Intraspecific variation in thermal tolerance and acclimation capacity in brook trout (*Salvelinus fontinalis*): physiological implications for climate change. *Physiological and Biochemical Zoology* 87: 15-29.

Strobel, A., Bennecke, S., Leo, E., Mintenbeck, K., Pörtner, H.O., and Mark, F.C. 2012. Metabolic shifts in the Antarctic fish *Notothenia rossii* in response to rising temperature and PCO_2. *Frontiers in Zoology* 9: 28.

Sutterlin, A.M., Henderson, E.B., Merrill, S.P., Saunders, R.L., and MacKay, A.A. 1981. Salmonid rearing trials at Deer Island, New Brunswick, with some projections on economic viability. *Canadian Technical Report on Fisheries and Aquatic Sciences* No. 1011, 28 pp.

Taylor, J.F., Waagbø, R., Diez-Padrisa, M., Campbell, P., Walton, J., Hunter, D., Matthew, C., and Migaud, H. 2015. Adult triploid Atlantic salmon (*Salmo salar*) have higher dietary histidine requirements to prevent cataract development in seawater. *Aquaculture Nutrition* 21: 18-32.

Thorstad, E.B., Fleming, I.A., McGinnity, P., Soto, D., Wennevik, V. and Whoriskey, F. 2008. Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. Norwegian Institute for Nature Research Special Report 36. 110 pp.

Ultsch, G.R. and Nordlie, F.G. 2019. The case for reporting PO_2 (partial pressure of oxygen), in addition to DO (dissolved oxygen), in studies of aquatic systems. *Comparative Biochemistry and Physiology, Part A* 235: 66-68.

Underwood, Z.E., Myrick, C.A., and Rogers, K.B. 2012. Effect of acclimation temperature on the upper thermal tolerance of Colorado River cutthroat trout *Oncorhynchus clarktii pleuriticus*: thermal limits of a North American salmonid. *Journal of Fish Biology* 80: 2420-2433.

Vargas-Chacoff, L., Regish, A.M., Weinstock, A., and McCormick, S.D. 2018. Effects of elevated temperature on osmoregulation and stress responses in Atlantic salmon *Salmo salar* smolts in fresh water and seawater. *Journal of Fish Biology* 93: 550-559.

Wall, A.E. and Richards, R.H. 1992. Occurrence of cataracts in triploid Atlantic salmon (*Salmo salar*) on four farms in Scotland. *Veterinary Record* 131: 553-557.

Waltz, E. 2017. First transgenic salmon sold. Nature 548: 7666. Available from URL: https://www.nature.com/news/first-genetically-engineered-salmon-sold-in-canada-1.22116.

Wehrly, K.E., Wiley, M.J., and Seelbach, P.W. 2003. Classifying regional variation in thermal regime based on stream fish community patterns. *Transactions of the American Fisheries Society* 132: 18-38.

Wendelaar Bonga, S.E. 1997. The stress response in fish. *Physiological Review* 77: 592-616.

Wringe, B.F., Jeffrey, N.W., Stanley, R.R.E., Hamilton, L.C., Anderson, E.C., Fleming, I.A., Grant, C., Dempson, B., Veinott, G., Duffy, S.J. and Bradbury, I. R. 2018. Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. *Nature: Communications Biology* 1: 108.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., and Smith, G.M. 2009. Mixed Effect Models and Extensions in Ecology with R. Springer, New York, NY. 579pp.

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