HYPOXIA TOLERANCE OF TRIPLOID BROOK CHARR,

SALVELINUS FONTINALIS

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ABSTRACT

Triploid salmonids are used in aquaculture and stocking programs for recreational fishing because they are reproductively sterile and therefore cannot breed in the wild. However, they appear to be more sensitive than diploids to environmental stressors. The objective of this study was to develop a better understanding of the hypoxia tolerance of triploids. I compared the acute hypoxia tolerance of sibling diploid and triploid brook charr that were acclimated to either moderate hypoxia (65% of air saturation) or normoxia (98% air saturation). Fish then underwent acute hypoxia trials, by slowly injecting nitrogen gas to displace oxygen and using loss of equilibrium (LOE) as the endpoint. Hypoxia-acclimated fish had a lower oxygen tension (PO_2) at LOE compared to fish acclimated to normoxia, regardless of ploidy. Ploidy did not affect time to LOE, but triploids had a higher PO_2 at LOE (i.e., were less hypoxia tolerant) compared to diploids. Potential predictors of hypoxia tolerance (hematocrit, blood glucose, blood lactate, relative ventricular mass, hepatosomatic index, condition factor, compact myocardium thickness, and interlamellar cell mass) did not provide conclusive results. This study has shown the capacity for hypoxia acclimation to improve triploid performance, although not to the same extent as for diploids.

DEDICATION

To my family, thank you for everything you do.

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1. INTRODUCTION

1.1 Aquaculture in Canada

The aquaculture industry is a vital part of New Brunswick's economy, with a farm gate value of $125 million and providing primary full-time equivalent employment to 495 people in 2020 (Government of New Brunswick, 2020). Atlantic salmon (*Salmo salar*) currently represent 90% of this production value in New Brunswick (Government of New Brunswick, 2020). Globally, Canada is ranked 4th in farmed salmon production, 15% of which comes from New Brunswick (Fisheries and Oceans Canada, 2020).

From hatching to smoltification, Atlantic salmon are reared in land-based freshwater facilities, after which they are placed in ocean net pens for 1–2 years, where they undergo most of their growth. While in these net pens, farmed salmon are at a higher risk of interaction with locally adapted and genetically distinct wild salmon populations (Glover et al., 2017). In instances of net pen failures or other issues leading to escaped fish, farmed salmon can outcompete, displace and breed with wild populations, leading to modifications of the wild Atlantic salmon genome (Glover et al., 2017; Wringe et al., 2018). This is because wild and hatchery reared fish differ in a multitude of ways, including growth rate, morphology, life history, behaviour, physiology, gene transcription and genetic markers (Glover et al., 2017). There is also a higher probability of genetic introgression in areas with low wild adult densities (Glover et al., 2012), and genetic changes to wild Atlantic salmon genomes as a result of interbreeding with farmed escapees have already been documented in Ireland (Clifford et al., 1998) and Canada (Bourret et al., 2011) causing global concerns.

With New Brunswick's wild Atlantic salmon population at an all-time low (Government of Canada, 2019) and listed as endangered since the early 2000s (Fisheries and Oceans Canada, 2019), the population is at an increased risk of further genetic introgression. In 2010, there were three net pen breaches in New Brunswick where a total of 184 000 farmed Atlantic salmon escaped (Fisheries and Oceans Canada, 2010) and recapture was not attempted. In August 2019, a net failure resulted in 1000 escapees from a site near Deer Island and only 53 of these fish were captured in the Magaguadavic fishway in St. George (Atlantic Salmon Federation, 2019). In 1992, almost 300 wild Atlantic salmon passed through this fishway, but in August 2019, only two wild individuals were present. This is just one local example displaying how escapes from salmon farms can quickly overwhelm native populations. In addition to outcompeting wild populations, there is concern that genetic introgression could lead to decreased resilience to environmental challenges. Major reductions in farmed escapees and sterility (via induced triploidy) of these escapees are two of the main ways to help address these issues (Glover et al., 2017).

1.2 Triploidy: Advantages and Pitfalls

Triploidy is the result of an organism having three sets of homologous chromosomes due to retention of the second polar body during cell division, specifically meiosis II. The resulting triploid zygote has two sets of maternal chromosomes and one set of paternal chromosomes. Triploidy can easily be induced at the commercial level by physical or chemical shock to fertilized eggs (McGeachy et al., 1995), timed precisely to disrupt meiosis II (~200°C-min post-fertilization), and preventing extrusion of the second

polar body (Piferrer et al., 2009). As a result of their additional genetic material, triploids either fail to produce gametes or produce aneuploid gametes that lead to unviable offspring (Li et al., 2016), therefore rendering them reproductively sterile (Benfey, 2001).

Due to their sterility, the use of triploids has been proposed as a way to prevent genetic introgression between farmed and native wild fish (Benfey, 2016). Triploids also have the added benefit of reduced gonadal growth, and with less energy being put toward gamete production, triploids (specifically females) have shown increased somatic growth and muscle pigmentation (Benfey 1999), resulting in a higher quality fillet. However, several studies have found triploids have higher mortality when enduring environmental challenges (Benfey, 1999; Altimiras et al., 2002; Hyndman et al., 2003a; Piferrer et al., 2009; Hansen et al., 2015; Sambraus et al., 2017) and perform poorly compared to diploids when exposed to chronically high temperature (Ojolick et al., 1995; Pepper et al., 2004; Hansen et al., 2015; Sambraus et al., 2017; Sambraus et al., 2018) and low oxygen (i.e., hypoxia) (Sambraus et al., 2018).

Increased instances of morphological deformities are another factor limiting the widespread commercial use of triploids. Skeletal (Madsen et al., 2000; Sadler et al., 2001; Fjelldal and Hansen, 2010) and lower jaw deformities (Sutterlin et al., 1987; Jungalwalla, 1991; Sutterlin and Collier, 1991), as well as cataracts leading to blindless and emaciation (Wall and Richards, 1992) and gill filament deformities, reducing gill surface area (Sadler et al., 2001) are commonly identified abnormalities of triploids.

One of the main physiological differences of triploids is their increased cell size (due to their increased genetic material) coupled with lower cell numbers to maintain normal organ and body size. Increased erythrocyte size and compensatory decrease in

erythrocyte number (Benfey, 1999) can affect the oxygen carrying capacity of the blood (Graham et al., 1985; Bernier et al., 2004), cardiac function (Verhille et al., 2013), and responsiveness to environmental stimuli (Benfey, 1999). These effects on whole-animal physiology are due to constraints placed on surface-mediated processes at the cellular level, potentially altering environmental tolerances and optima (Benfey and Devlin, 2018). However, despite these disadvantages, an economic modelling study showed that growing triploids can be more profitable than farming diploids (Berrill et al., 2012), but it is crucial to improve their performance under suboptimal conditions.

1.3 Environmental Challenges of Fishes

As climate change continues to impact ecosystems globally, both farmed and wild fish are experiencing more severe and longer lasting environmental stressors (Corkum and Gamperl, 2009; Vaquer-Sunyer and Duarte, 2011; Stehfest et al., 2017; Oldham et al., 2019; Brauner and Richards, 2020; Strowbridge et al., 2021). For instance, high temperatures and hypoxia are becoming increasingly common in aquatic environments (Vigen, 2008; Burt et al., 2012; Solstorm et al., 2018; Brauner and Richards, 2020). In addition to gradual increases in temperature and hypoxia, heat waves/spikes will be exacerbated as a result of anthropogenic climate change (Schmidt and Boyd, 2016). This combination of more frequent heat waves and gradual temperature increases will lead to water temperature and oxygen levels that are stressful and potentially lethal (Reid et al., 2019). This is because sustained chronic stress may exceed the capabilities of fish to maintain homeostasis, which can disrupt many functions, particularly growth and reproduction (Billard et al., 1981).

Increased temperature raises metabolic costs and limits oxygen availability, exposing all aquatic ectotherms to metabolically restricting conditions at all life stages (Jonsson and Jonsson, 2009; Sergeant et al., 2017; Breitburg et al., 2018). This aligns with the "oxygen and capacity-limited thermal tolerance" concept of temperature increase above an animal's thermal optimum resulting in reduced aerobic scope due to a greater proportion of the available oxygen being needed to support standard metabolism (Farrell, 2016; Pörtner et al., 2017). Given the pressing nature of anthropogenic climate change, a better understanding of these two environmental stressors is crucial (Stehfest et al. 2017; Reid et al., 2019; Brauner and Richards 2020; Gamperl et al. 2020).

As such, in the past decade there has been an increase in research focusing on warmer temperature and its effects on the physiology of fishes (Anttila et al., 2015; McBryan et al., 2016; Sambraus et al., 2017; Tunnah et al., 2017; Benfey and Devlin, 2018; Sambraus et al., 2018; Gamperl et al., 2020; Jensen and Benfey, 2022). A number of these studies investigated the effects of temperature acclimation (Anttila et al., 2015; McBryan et al., 2016; Sambraus et al., 2017; Sambraus et al., 2018; Gamperl et al., 2020; Jensen and Benfey, 2022), but few of them have included ploidy effects (Sambraus et al., 2017; Sambraus et al., 2018; Jensen and Benfey, 2022).

1.4 Hypoxia: Tolerance and Acclimation

For salmonids, growth, digestion, and reproduction are fueled by energy generated via aerobic metabolism (Claireaux and Chabot, 2016). However, at warmer temperatures, fish often experience a drop in arterial oxygen tension (PO_2), causing a reduction in oxygen availability to mitochondria, and reducing the ability of fish to

maximize oxygen uptake from the water (Pörtner, 2001). This is reflected by changes in aerobic scope, which is calculated as the difference between the maximum metabolic rate (MMR) and standard metabolic rate (SMR) and is a valuable tool for examining the effects of environmental stressors on fish physiology (Claireaux and Lefrançois, 2007; McKenzie et al., 2016). Once the limiting oxygen saturation required for basic needs is reached, aerobic scope is reduced (Richards et al., 2009; Norin and Clark, 2016) and fish are forced to limit all non-essential activities in order to put energy toward immediate survival (Burt et al., 2013; Eliason and Farrell, 2016; Remen et al., 2016).

In a 2001 review, Pörtner noted that aerobic scope is the first physiological process limited at high and low temperatures, and that at temperatures outside an animal's thermal optimum, aerobic scope is reduced, becoming a limiting factor for survival. The thermal optimum of most salmonids generally ranges from 6–20°C (Jensen et al., 1989), but a recent study by Tunnah et al. (2017) found water temperatures in a local New Brunswick river ranged from 20–27°C in the summer months, providing further support for the need to investigate environmental stressors, and even more so for researchers studying triploids and how they respond to stressful environmental conditions.

Many studies have investigated temperature and the physiological consequences fish endure when exposed to thermal stress (Anttila et al., 2015; McBryan et al., 2016; Sambraus et al., 2017; Tunnah et al., 2017; Benfey and Devlin, 2018; Sambraus et al., 2018; Gamperl et al., 2020; Jensen and Benfey, 2022). Lately, there has been even more focus toward how temperature fluctuations can affect a fish's hypoxia tolerance, essentially a fish's ability to cope with low oxygen availability in the water. Past studies

have found warm temperature acclimation increases hypoxia tolerance in brook charr (*Salvelinus fontinalis*) (Jensen and Benfey, 2022), Atlantic salmon (Sambraus et al., 2017), and killifish (*Fundulus heteroclitus*) (McBryan et al., 2016). There have been a few studies looking at hypoxia tolerance of triploids, with Scott et al. (2015) and Benfey and Devlin (2018) finding that across many strains, triploid rainbow trout (*Oncorhynchus mykiss*) were less hypoxia tolerant than diploids. Similar findings by Jensen and Benfey (2022) indicate that although temperature acclimation improved triploid hypoxia tolerance, they still had a lower tolerance than diploids.

Researchers have also looked at using hypoxia acclimation to improve hypoxia tolerance. In instances where hypoxia acclimation has been investigated, most studies have looked at short-term acclimation (Mitrovic et al., 2009; Fu et al., 2011; Oldham et al., 2019) or diel hypoxia–normoxia cycling (Remen et al., 2012; Yang et al., 2013; Dan et al., 2014; Hansen et al., 2015; Williams et al., 2019), with only a few studies having longer hypoxia acclimation periods ranging from 14-33 days (Sollid et al., 2003; Remen et al., 2013; Dan et al., 2014).

There has been less research investigating chronic moderate hypoxia acclimation as a tool for improving hypoxia tolerance of triploids. Most triploid hypoxia research has focused on the effects of water temperature (Sambraus et al., 2017; Benfey and Devlin, 2018; Jensen and Benfey, 2022) and energy, feed intake and production performance (Hansen et al., 2015; Sambraus et al., 2018). As previously mentioned, it seems that triploids are often outperformed by their diploid counterparts and Brauner and Richards (2020) stress the importance of further investigation to improve triploid performance

under hypoxic conditions. Past and current research indicates a knowledge gap regarding the use of chronic hypoxia acclimation to improve triploid hypoxia tolerance.

1.5 Assessment of Hypoxia Tolerance

Hypoxia tolerance can be assessed in many ways, but Brauner and Richards (2020) state that critical oxygen tension (P_{crit}) or the oxygen tension (PO_2) at loss of equilibrium (LOE) and time to reach LOE are among the best ways to quantify hypoxia tolerance. LOE is defined as a fish's inability to maintain dorso-ventral orientation for more than five seconds and is commonly used as a physiological endpoint in salmonid studies (Brauner and Richards, 2020). A lower P_{crit} is generally accepted as an indication of improved hypoxia tolerance; Speers-Roesch et al. (2012) determined that this is associated with greater arterial oxygen content, and therefore potentially improving oxygen delivery to tissues. Dissolved oxygen in such studies is expressed as oxygen tension because oxygenation of the blood is dependent on a pressure-based diffusion gradient (Evans et al., 2005) and PO_2 is therefore a more accurate predictor of hypoxia tolerance in fish (Ultsch and Nordlie 2019), with both a lower PO_2 at LOE and a longer time to LOE indicating improved hypoxia tolerance. The physiological mechanisms behind hypoxia-induced LOE are not fully understood, but it is likely due to a decrease in ATP production during hypoxia, leading to gradual loss of physiological function (Brauner and Richards, 2020).

1.6 Physiological Responses to Hypoxia

Several parameters can be investigated to understand the potential physiological response to suboptimal environmental conditions; these include whole blood or blood

plasma measurements and morphological changes. Since triploids have a fewer number of larger erythrocytes, the percentage of total blood volume occupied by cells (hematocrit) is the same as in diploids (Benfey, 1999). As such, Benfey and Biron (2000) found increases in hematocrit of diploid and triploid rainbow trout and brook charr were not significantly different when under stressful conditions. Non-significant differences in increased plasma glucose and cortisol levels were also seen. Glucose is an important energy source for metabolism in all organisms, and when enduring stress is often produced to immediately provide energy to evade or adjust to the stressor (Beyea et al., 2005). This elevation of blood glucose during stress is due to elevated blood catecholamine levels, which is part of the primary stress response in fish (Pottinger, 1998). Blood lactate increases occur due to respiratory activity under anaerobic conditions in which glycogen stores are depleted and lactate accumulates in the muscle tissues before being released to the blood when the animal returns to aerobic respiration (Milligan and Girard, 1993). It should be noted that increased lactate levels alone may not be an indication of a stress response, but reflects tissue requirements for oxygen exceeding supply (Pottinger, 1998). Work by Hyndman et al. (2003a) found that resting glucose and lactate concentrations (mean ± 1 S.E.M) were similar; 4.6 ± 0.5 and 5.2 ± 0.5 mmol for diploids and triploids, respectively, but glucose concentrations increased significantly only in diploids (8.1 ± 1.0 mmol) following exhaustive exercise. In response to hypoxia, Atlantic salmon plasma lactate levels increased significantly higher than in normoxia (Oldham et al., 2019). Similarly, Fu et al. (2011) found elevated plasma lactate concentrations in hypoxia-acclimated goldfish (*Carassius auratus*).

Cardiac remodeling is among the most frequently researched stress-induced morphological changes in fish. This is because the heart's ability to supply blood to all metabolic active tissues is a primary indicator of stress tolerance (Imbrogno et al., 2022), and generally indicates adaptive response limitations of an organism. The salmonid ventricle has two myocardial layers: the compact (outer) layer that consists of circumferentially arranged cardiomyocytes and the spongy (inner) layer that has an arrangement of muscular trabeculae (Pieperhoff et al., 2009). The compact myocardium is suppled fully oxygenated blood via coronary circulation, whereas the spongy myocardium must extract oxygen from venous blood (Farrell et al., 2009). The proportion of the ventricle comprised of compact myocardium varies among species and also changes with cardiac growth during development; however, in the most athletic of fishes, compact myocardium rarely exceeds 60% of ventricular mass (Farrell et al., 2009). Since it composes the inner ventricle layer, the spongy myocardium is routinely exposed to lower oxygen levels compared to the compact myocardium and changes in oxygen availability can alter the performance of these two layers in different ways (Imbrogno et al., 2022). For example, Roberts et al. (2021) found that spongy myocardium did not benefit from hypoxia acclimation, whereas compact myocardium contractile performance was doubled.

Cardiac output is defined as heart rate × stroke volume, and since arterial blood is fully saturated with oxygen, increasing cardiac output allows fish to transfer substantially more oxygen to their tissues (Farrell et al., 2009). Stroke volume is dependent on ventricle size, with a larger ventricle correlating to increased stroke volume, and thus increased cardiac output. Generally, salmonids are more likely to increase stroke volume

than heart rate when stressed (Farrell et al., 2009). As such, relative ventricular mass (RVM; relative to body mass) is another parameter studied when discussing stress-induced responses in fish, with an increase in RVM (i.e., increases in spongy and/or compact myocardium) indicating better hypoxia tolerance (Klaiman et al., 2011). It is advantageous for fish that inhabit areas with environmental fluctuations (such as salmonids) to have some capacity for cardiac remodeling, and having a larger RVM helps to increase contractile function by increasing the number of contractile units (i.e., increasing cardiac output) (Graham and Farrell, 1989). One study looking at rainbow trout found a decrease in compact myocardium thickness in fish acclimated to cold temperatures, and an increase in compact myocardium thickness when acclimated to warmer temperatures (Klaiman et al., 2011). Other researchers found similar temperature-dependent effects on both RVM and compact myocardium in rainbow trout (Graham and Farrell, 1989). In a 2004 review, Gamperl and Farrell discuss cardiac plasticity of fish in response to hypoxia acclimation, noting that crucian carp (*Carassius carassius*) (Paajanen and Vornanen, 2003), European flounder (*Platichthys flesus*) (Lennard and Huddart, 1992), and ocean pout (*Zoarces americanus*) (Driedzic et al., 1985) all underwent morphological and physiological adjustments to improve performance under hypoxic conditions. More recent work found hypoxia-induced decline in ventricular output (Roberts, 2016).

Gills also play an integral role in a fish's response to hypoxia, with the gill lamellae being the primary site for gas exchange. Each lamella is made up of two epithelial layers separated by pillar cells, and oxygen is taken up by erythrocytes from the water flowing between the lamellae. The space between adjacent lamellae is filled with

cells to create the interlamellar cell mass (ILCM). A smaller ILCM corresponds to an increased respiratory surface area, and thus a better ability to take up oxygen from the water. Studies have shown that acclimation to hypoxia or high temperature causes a decrease in ILCM size in goldfish (Sollid et al., 2005; Mitrovic et al., 2009), crucian carp (Sollid et al., 2003, 2005), and mangrove killifish (*Kryptolebias marmoratus*) (Ong et al., 2007). While a larger respiratory surface area improves gas exchange, it also increases the surface area for diffusion of ions and therefore increases the need for energetically expensive ion regulation that is supported by an increased SMR; this is referred to as "the osmorespiratory compromise" (Nilsson, 2007).

1.7 Brook Charr as Research Animal

Brook charr were used in this study as a model salmonid closely related to the heavily farmed Atlantic salmon but smaller in size and better suited for the aquatic facility at the University of New Brunswick, where rearing post-smolt Atlantic salmon is not feasible. In addition to commercial relevance, brook charr are a prominent angling fish, and are used in stocking programs at both the provincial and national level. Thus, having a better understanding of brook charr hypoxia tolerance (regardless of ploidy) has several benefits, on both a commercial and recreational scale. Given that maturing diploid females develop much larger ovaries than triploid females, juveniles were used to limit any confounding effects of using older fish with differing gonadal sizes.

1.8 Aim of Research

The objective of my MSc research was to develop a better understanding of the biological requirements of triploids (specifically hypoxia tolerance), and thereby provide

advice to the aquaculture industry on how to improve triploid performance. More specifically, I tested the hypothesis that chronic moderate hypoxia acclimation improves hypoxia tolerance in brook charr, with the prediction that hypoxia-acclimated diploids will have the highest hypoxia tolerance (as measured by PO_2 at LOE and time to LOE), followed by hypoxia-acclimated triploids, then normoxia-acclimated diploids and finally normoxia-acclimated triploids. Hypoxia tolerance studies are relevant to the aquaculture industry since both land-based hatchery facilities (Guinea and Fernandez, 1997) and ocean net pens (Guinea and Fernandez, 1997; Burt et al., 2012; Stehfest et al., 2017) for Atlantic salmon often experience hypoxic conditions. Several recent studies have expressed the importance of developing a better understanding of hypoxic events and their impacts for salmonid aquaculture (Remen et al., 2012; Tunnah et al., 2017; Williams et al., 2019; Gamperl et al., 2020). My research built on the work of current and past students of the Benfey lab. This lab has done several studies on temperature and hypoxia tolerance of triploid brook charr but has not investigated chronic moderate hypoxia acclimation as a tool for improving their hypoxia tolerance.

2. METHODS

2.1 Rearing Information

This research was approved by the UNB Animal Care Committee (Animal Use Protocol 21-003), following the guidelines of the Canadian Council on Animal Care, and conducted in the University of New Brunswick aquatic facility using brook charr produced in December 2019 from an in-house breeding program. Triploidy was induced via hydrostatic pressure shock treatment at 65.5 mPa for 5 minutes, beginning 200ºC-min post-fertilization (model TRC-APVM, TRC Hydraulics Inc., Dieppe, NB, Canada) to 50% of the eggs. The remaining eggs were retained as diploid controls. All embryos and larvae were incubated in the dark until yolk-sac absorption in March 2020. The fry were then placed in 56 L circular flow-through tanks with dechlorinated municipal water at ambient temperature and reared according to standard salmonid protocols (Jobling et al., 2010).

In March 2021, juveniles were transferred into 56 L tanks in two identical 18-tank recirculating aquaculture systems in separate rooms. Each tank was stocked with 20 fish and supplied with dechlorinated municipal water at a flow rate of 4 L/min. Ploidy alternated between adjacent tanks, with 9 tanks of each ploidy in each system. One system was set as the chronic hypoxia system (target of 70% of air saturation) and the other system was set as the normoxia control system (target of 100% of air saturation). Both systems were maintained at 13.5ºC and a 12L:12D photoperiod. The initial intent was to have both systems at 15°C, the thermal optimum for rearing diploid brook charr (Smith and Ridgway, 2019); however, this was not feasible within the systems, and

13.5°C was used. To obtain chronic hypoxia, the biofilter had to be bypassed and make-up water for the system was increased to 20 L/min, representing a switch from full recirculation to partial flow-through. The same modifications were made to the normoxia system to maintain comparable systems. Water quality parameters (ammonia, nitrite, and nitrate) were measured weekly in both systems. Each recirculating system's oxygen control unit was then used to maintain dissolved oxygen levels within 5% of target values through the automated activation of aeration systems in all 18 tanks. Prior to the experiment, all fish were anesthetized, measured (body mass (g) and fork length (cm)), and had either their left (hypoxia) or right (normoxia) pectoral fin clipped for identification purposes. Throughout this study, a stock solution of 50 g benzocaine (ethyl 4-aminobenzoate, 98%; catalogue number 112909, Sigma-Aldrich, St. Louis, MO, United States) dissolved in 1 L of 95% ethanol was used for anesthesia (diluted to 1% in tank water) and euthanasia (diluted to 10%).

Fish were acclimated to either chronic hypoxia or normoxia for 7 weeks beginning on April 19th, 2021. During this acclimation period, fish were fed 1% of tank biomass of 4 mm salmonid pellets (Corey Feed Mills Ltd., Fredericton, NB, Canada) by hand once daily between 0800h and 1000h. This was reduced to 0.5% of tank biomass during the acute hypoxia trials, with feed withheld for 24 hours from the designated tanks prior to a given trial. This is a standard aquaculture practice to avoid regurgitation and reduce SMR of the fish, allowing them to better cope with the stress of the acute hypoxia trials. Temperature and dissolved oxygen levels were measured daily for the duration of the acclimation period and the acute hypoxia trials (April 19th – June 25th) using a

handheld oxygen and temperature probe (model ProODOTM, YSI Inc., Yellow Springs, OH, United States) between 1300h and 1500h.

2.2 Acute Hypoxia Trials

Acute hypoxia trials began on June 7th, 2021 and concluded on June 25th, 2021. One trial was carried out each day for 18 days (June 7th-June 15th and June 17th-25th) with each trial starting at approximately 0800h. No acute hypoxia trial was done on June 16th to allow for baseline blood collection as described below. A convenience sample protocol was used to select three diploids and three triploids from each of the two acclimation groups (hypoxia and normoxia) resulting in a total of 12 fish for each trial and 216 fish in total over the 18-day trial period. The four treatment groups are denoted as 2N70 (hypoxia-acclimated diploids), 3N70 (hypoxia-acclimated triploids), 2N100 (normoxia-acclimated diploids), and 3N100 (normoxia-acclimated triploids).

Acute hypoxia trials were conducted in a 44 L stainless steel water bath (90 cm × 28 cm × 17.5 cm deep) set to 13.5ºC and filled with ~40 L of dechlorinated water from the same water source as the rearing tanks (Figure 1). The water bath was fitted with a clear plexiglass lid to prevent fish from escaping. Trials started with dissolved oxygen at target values of either 70% or 100% of air saturation, alternating daily. Fish were held in the water bath for one hour before the acute hypoxia trial began, as is consistent with previous work in the Benfey lab (Benfey and Devlin, 2018; Jensen and Benfey, 2022).

Oxygen depletion at a rate of 1%/min was achieved by injecting nitrogen gas via micro bubble diffuser (model MBD600, Valox Ltd., Fredericton, NB, Canada) to displace the oxygen and using loss of equilibrium (LOE) as the endpoint (Brauner and Richards,

2020). LOE was defined as a fish being unable to maintain dorso-ventral orientation for five seconds. The rate of deoxygenation was maintained by manually adjusting the nitrogen regulator and using the handheld oxygen meter to monitor the oxygen levels. As fish lost equilibrium, they were removed from the water bath via dip net and placed in individual recovery buckets containing 3 L of dechlorinated, room temperature water (~15 ºC). Time to LOE and dissolved oxygen (% of air saturation) at LOE were recorded for each fish. Dissolved oxygen was later converted to oxygen tension (PO_2; kPa) using PreSens Oxygen Calculator v3.1.1 (PreSens Precision Sensing GmbH; Regensburg, Germany).

2.3 Blood Sampling

Once all fish in the trial had lost equilibrium, they were individually anesthetized, measured (fork length and body mass), and their acclimation group was recorded according to their fin clip. This processing was done in the same order as they lost equilibrium. Using a lithium heparinized needle and 1 mL syringe, a blood sample was taken via caudal vasculature and transferred into a 5 mL Falcon tube (Corning Science; catalogue number 352054, Glendale, AZ, United States). Whole blood was used to make blood smears for later ploidy confirmation and to measure hematocrit (Thermo Fisher Scientific; catalogue number 22-362566), glucose (FreeStyle Lite) and lactate (L+ Lactate Test Strips; catalogue number 40813, Lactate Plus Analyzer; catalogue number 62624, Sports Resource Group Inc., Exeter, NH, United States). The remaining blood was centrifuged (Hermle Z 360 K Refrigerated Centrifuge, at 4.3×10^3 RPM for 10 minutes) and the plasma frozen for use in future research projects.

As noted above, at the mid-way point (June 16th) of the acute hypoxia trials, 12 fish per acclimation group (6 of each ploidy) were anesthetized, and blood was taken and processed as outlined above. This was done to obtain baseline data for hematocrit, glucose, and lactate levels from fish not subjected to the acute hypoxia protocol.

2.4 Dissections

Following blood collection, fish that underwent the acute hypoxia trials were euthanized and dissected to remove and weigh the ventricle, liver, and gonads. The first two gill arches on the left side were also removed but not weighed. Ventricles and gills were fixed and stored in 10% buffered formalin (Thermo Fisher Scientific; catalogue number SF994, Waltham, MA, United States) for later histology to quantify compact myocardium thickness and interlamellar cell mass (ILCM) respectively. Weight measurements were used to calculate Fulton's condition factor (Formula 1), relative ventricular mass (RVM; Formula 2), hepatosomatic index (HSI; Formula 3) and gonadosomatic index (GSI; Formula 4). The sex of each fish was determined by visual observation of the gonads.

Formula 1: $Condition\ Factor = \frac{Body\ Mass\ (g)}{Fork\ Length\ (cm)^3} \times 100$

Formula 2: $RVM = \frac{Ventricle\ Mass\ (g)}{Body\ Mass\ (g)} \times 100$

Formula 3: $HSI = \frac{Liver\ Mass\ (g)}{Body\ Mass\ (g)} \times 100$

Formula 4: $GSI = \frac{Gonad\ Mass\ (g)}{Body\ Mass\ (g)} \times 100$

2.5 Ploidy Confirmation

Ploidy was confirmed by imaging blood smears at 40× using a camera attachment (model MU900, AmScope, Irvine, CA, United States) connected via USB to a computer with imaging software (AmLite for Mac version 3.7). Images were taken from five fields of view of each blood smear, and five erythrocytes were measured in each image, resulting in 25 measurements for each fish. Average erythrocyte length was used to assign ploidy (Benfey et al., 1984).

2.6 Histology

Prior to tissue embedding, all ventricle samples were transferred from 10% buffered formalin to 75% ethanol for 24 hours and rinsed in a second 75% ethanol solution for an additional 24 hours. They were then embedded using paraffin wax (Shandon Embedding Center; catalogue number B64110040, Fisher Scientific, Waltham, MA, United States) and cut into 7 μm sagittal sections using a microtome (Leica RM2235; catalogue number 8226-30-1009, GMI Inc., Ramsey, MN, United States) and transferred onto slides. Sections were stained using the hematoxylin and eosin protocols established by the microscopy staff at UNB (Appendix I) and mounted under a coverslip using Distyrene Plasticizer Xylene. Mounted sections were imaged at 10× using a light microscope (Leica DM R upright microscope), and compact myocardium thickness (μm) was measured using ImageJ software (version 1.53; Abràmoff et al., 2004). Five measurements were taken for each ventricle and averaged for a final measurement of compact myocardium thickness for each fish.

Dissected gills were transferred from 10% buffered formalin to 75% ethanol for 24 hours, decalcified in hydrochloric acid (100% Cal-Ex Decalcifier, Fisher Chemical; model number MFCD00211743) for 24 hours, followed by two more rinses in 75% ethanol, each lasting 24 hours. Gills were then embedded and sectioned following the protocols outlined above. Gill sections were stained using the Masson's trichome protocols established by the microscopy staff at UNB (Appendix I) and mounted as above. Gill imaging was done as outlined above (except measured at 20× instead of 10×). Lamellar and ILCM lengths were measured from their base to their tips (μm) and relative ILCM length was then calculated (Formula 5; average of five measurements per gill).

Formula 5: $Relative\ ILCM = \frac{Average\ ILCM\ height\ (\mu m)}{Average\ filament\ length\ (\mu m)} \times 100$

2.7 Statistical Analysis

The statistical approach was fashioned after analyses of Jensen and Benfey (2022) for a similar study. All statistical analyses were performed in R version 4.0.2 (R Core Team, 2020). Gonadosomatic index was excluded from the analysis as there were too many fish with undetermined sex. Separate general linear models (GLMs) were constructed to test the effects of ploidy, oxygen acclimation, and their interactions on body mass, fork length, condition factor, RVM, HSI, hematocrit, glucose, lactate, ILCM, and compact myocardium thickness. Additional GLMs were constructed testing the effects of ploidy, oxygen acclimation, treatment (baseline or stressed) and their interactions on hematocrit, glucose, and lactate levels. Post-hoc Tukey's tests were performed in the case of significant findings from any of these GLMs.

Two separate linear mixed-effects models (lme4 package; Bates et al., 2015) were then used to test the effects of ploidy, oxygen acclimation, oxygen starting level, condition factor, RVM, HSI, hematocrit, ILCM, and compact myocardium thickness on PO_2 at LOE and time to LOE. Tank and trial (=day) were included as random factors. Each time the model was run, non-significant interactions and main effects were removed using backward stepwise selection, until only significant effects remained (Appendix II). The final (reduced) results were plotted to allow for visualization of any remaining effects. PO_2 at LOE and compact myocardium thickness data were log-transformed before being included in the model as they did not meet the assumption of homoscedasticity. Fish with missing values were excluded from the analysis. A significance level of $\alpha = 0.05$ was used for all tests.

Figure 1: Water bath setup for acute hypoxia trials. Oxygen depletion was achieved by bubbling compressed nitrogen gas through two micro bubble diffusers. Temperature and dissolved oxygen levels were continuously monitored via a handheld probe.

3. RESULTS

Temperature in the fish tanks rose by approximately 1°C over the course of the 18-day experiment with no consistent difference between systems (normoxia versus hypoxia; Figure 2). This temperature increase was due to a heat wave in early June that increased air temperature in the Aquatic Facility and incoming water temperature. This was accounted for by including "day" as a random factor in the linear mixed-effects modeling, and there was nothing to indicate hypoxia tolerance was affected by this increase in temperature. Dissolved oxygen levels were maintained close to the target values of 100% (98 ± 4.6%; mean ± SD) and 70% (65 ± 3.3%; mean ± SD) of air saturation, respectively (Figures 3–4). Ammonia (hypoxia: 0.02 ± 0.05; normoxia: 0.02 ± 0.03; mean ± SD), nitrite (hypoxia: 0.01 ± 0.01; normoxia: 0.03 ± 0.01; mean ± SD) and nitrate (hypoxia: 0.2 ± 0.29; normoxia: 0.2 ± 0.33; mean ± SD) levels were consistent between the systems. The rate of oxygen depletion was consistent during the acute hypoxia trials (Figure 5).

Erythrocyte size measurements revealed two discrete populations representing diploids (194 ± 6.7 pixels; mean ± SD) and triploids (248 ± 12.0 pixels; mean ± SD) (Figure 6). All but two fish were confirmed to be of the assumed ploidy, with the two exceptions being presumed diploids that were determined to be triploid. This resulted in a total of 106 diploids and 110 triploids; however, since some fish were excluded from analyses because of missing data points, the final mixed-effects modelling included 188 fish (96 diploids and 92 triploids).

The average initial starting dissolved oxygen level of the acute hypoxia trials were 87% and 70% for the trials intended to start at 100% and 70% respectively. Oxygen in the water bath was increased by aeration, and unfortunately this resulted in less than 100% for the trials days intended to start at normoxia. Approximate time between going into recovery post-LOE and being processed was 30 minutes for each fish. There were no mortalities during the acclimation period and the acute hypoxia testing/recovery.

Fish that underwent the acute hypoxia trials had higher glucose and lactate levels compared to untested (baseline) fish, but they did not differ in hematocrit (Table 1; Figure 7), The overall model for glucose was not significant, but it was approaching significance (*p*-value = 0.068). Irrespective of whether subjected to the acute hypoxia trials or not, hypoxia-acclimated fish had higher hematocrit than normoxia-acclimated fish, although the overall model for hematocrit was not significant (Table 1; Figure 7).

Ploidy did not affect body mass (Table 2; Figure 8). Although the overall model for fork length was not significant, it was approaching significance *(p*-value = 0.060) and the separate GLM showed triploids were significantly longer than diploids (Table 2; Figure 8). Triploids also had a lower condition factor than diploids (Table 2; Figure 8). Cardiac metrics (RVM and compact myocardium thickness) were also not affected by ploidy; however, triploids had relatively smaller livers (lower HSI) and reduced respiratory surface area (larger ILCM) compared to diploids (Table 2; Figure 9). Although the overall model for glucose was not significant, triploids also had lower blood glucose levels than diploids, but they did not differ in hematocrit or blood lactate levels (Table 2; Figure 10). The only metric affected by oxygen acclimation level was RVM (higher in fish acclimated to normoxia than fish acclimated to hypoxia (Figure 9) and

there were no significant interactions between ploidy and oxygen acclimation level for any of these metrics.

Acute hypoxia trials that started at average 87% air saturation resulted in fish having a longer time to LOE compared to trials starting at average 70% air saturation (Table 3; Figure 11) but there was no effect of oxygen starting point on PO_2 at LOE. Hypoxia-acclimated fish had a lower PO_2 at LOE (Table 4; Figure 12) compared to fish acclimated to normoxia, regardless of ploidy. Ploidy did not affect time to LOE, but triploids had a higher PO_2 at LOE compared to diploids (Table 4; Figure 12). For trials starting at 70% air saturation, higher RVM resulted in a shorter time to LOE (Table 3; Figure 13). Plotting the relationship between hematocrit and PO_2 at LOE showed that apart from diploids acclimated to normoxia, fish with higher hematocrit had a higher PO_2 at LOE (Table 4; Figure 14).

Table 1: Results of general linear models (using Type II SS) testing the effects of ploidy (diploid or triploid), oxygen acclimation (normoxia or hypoxia), treatment (baseline or stressed) and their interactions on hematocrit ($R^2 = 0.03$, *p*-value = 0.311), glucose ($R^2 = 0.05$, *p*-value = 0.068), and lactate ($R^2 = 0.52$, *p*-value = <0.001) of juvenile brook charr (*Salvelinus fontinalis*). Parenthesis following categorical variables indicate reference levels and bold values indicate a significant relationship (p<0.05).

Source of variation	df	Coeffi-cient	Coefficient SE	MS	F-value	*p*-value
Hematocrit						
Treatment (Stressed)	1	-0.20	2.80	39.19	0.93	0.336
Ploidy (Triploid)	1	-0.85	3.75	57.42	1.36	0.244
O_2 Acclimation (Normoxia)	**1**	**-2.69**	**3.75**	**164.46**	**3.90**	**0.050**
Treatment × Acclimation	1	2.30	3.96	23.99	0.57	0.451
Treatment × Ploidy	1	0.98	3.95	3.50	0.08	0.773
Acclimation × Ploidy	1	-1.72	5.30	61.55	1.46	0.228
Treatment × Acclimation × Ploidy	1	-0.34	5.59	0.16	<0.001	0.951
Residuals	232			42.16		
Glucose						
Treatment (Stressed)	**1**	**5.07**	**2.23**	**239.33**	**8.90**	**0.003**
Ploidy (Triploid)	1	0.72	2.99	84.40	3.14	0.078
O_2 Acclimation (Normoxia)	1	1.63	2.99	0.24	<0.001	0.925
Treatment × Acclimation	1	-1.51	3.16	13.29	0.49	0.483
Treatment × Ploidy	1	-1.88	3.16	20.29	0.76	0.386
Acclimation × Ploidy	1	-0.32	4.23	2.57	0.10	0.758
Treatment × Acclimation × Ploidy	1	-0.11	4.46	0.02	<0.001	0.981
Residuals	232			26.89		
Lactate						
Treatment (Stressed)	**1**	**6.41**	**0.88**	**1066.30**	**254.356**	**<0.0001**
Ploidy (Triploid)	1	-0.42	1.18	0.10	0.019	0.890
O_2 Acclimation (Normoxia)	1	-0.47	1.18	4.30	1.018	0.314
Treatment × Acclimation	1	0.87	1.25	2.30	0.557	0.456
Treatment × Ploidy	1	0.56	1.25	0.60	0.154	0.695
Acclimation × Ploidy	1	0.28	1.67	0.20	0.037	0.849
Treatment × Acclimation × Ploidy	1	-0.42	1.76	0.20	0.059	0.809
Residuals	232			4.20		

Table 2: Results of general linear models (using Type II SS) testing the effects of ploidy (diploid or triploid), oxygen acclimation (normoxia or hypoxia), and their interactions on body mass (R^2 = 0.01, *p*-value = 0.576), fork length (R^2 = 0.03, *p*-value = 0.060), condition factor (R^2 = 0.09, *p*-value = <0.001), relative ventricular mass (R^2 = 0.04, *p*-value = 0.035), hepatosomatic index (R^2 = 0.006, *p*-value = 0.01), hematocrit (R^2 = 0.02, *p*-value = 0.172), glucose (R^2 = 0.02, *p*-value = 0.159), lactate (R^2 = 0.01, *p*-value = 0.697), interlamellar cell mass (R^2 = 0.05, *p*-value = 0.013) and compact myocardium thickness (R^2 = 0.01, *p*-value = 0.729),of juvenile brook charr (*Salvelinus fontinalis*). Parenthesis following categorical variables indicate reference levels and bold values indicate a significant relationship (p<0.05).

Source of variation	df	Coefficient	Coefficient SE	MS	F-value	*p*-value
Body Mass						
Ploidy (Triploid)	1	-2.01	2.86	70.59	0.32	0.572
O_2 Acclimation (Normoxia)	1	1.58	2.88	325.83	1.48	0.225
Ploidy × O_2 Acclimation	1	1.73	4.04	40.36	0.18	0.669
Residuals	212			220.00		
Fork Length						
Ploidy (Triploid)	**1**	**0.41**	**0.22**	**9.63**	**7.16**	**0.008**
O_2 Acclimation (Normoxia)	1	0.08	0.23	0.45	0.34	0.562
Ploidy x O_2 Acclimation	1	0.02	0.32	0.00	0.00	0.957
Residuals	212			1.34		
Condition Factor						
Ploidy (Triploid)	**1**	**-0.10**	**0.03**	**0.38**	**20.57**	**<0.001**
O_2 Acclimation (Normoxia)	1	0.01	0.03	0.019	1.03	0.310
Ploidy × O_2 Acclimation	1	0.03	0.04	<0.001	0.50	0.482
Residuals	212			0.02		
Relative Ventricular Mass						
Ploidy (Triploid)	1	-0.00	0.00	0.01	1.47	0.314
O_2 Acclimation (Normoxia)	**1**	**0.01**	**0.00**	**0.05**	**6.82**	**<0.001**
Ploidy × O_2 Acclimation	1	0.00	0.00	0.00	0.24	0.642
Residuals	212			0.01		
Hepatosomatic Index						
Ploidy (Triploid)	**1**	**-0.26**	**0.10**	**3.50**	**11.84**	**<0.001**
O_2 Acclimation (Normoxia)	1	-0.08	0.10	0.27	0.90	0.343
Ploidy x O_2 Acclimation	1	0.02	0.15	<0.001	0.01	0.910
Residuals	212			0.30		
Hematocrit						
Ploidy (Triploid)	1	0.13	1.25	43.51	1.03	0.312
O_2 Acclimation (Normoxia)	1	-0.40	1.26	112.72	2.66	0.104
Ploidy × O_2 Acclimation	1	-2.06	1.77	57.29	1.35	0.246
Residuals	212			42.30		
Glucose						
Ploidy (Triploid)	**1**	**-1.57**	**0.98**	**134.56**	**5.20**	**0.024**
O_2 Acclimation (Normoxia)	1	0.122	0.99	0.69	0.03	0.871
Ploidy × O_2 Acclimation	1	-0.02	1.39	0.01	<0.001	0.989
Residuals	212			25.86		
Lactate						
Ploidy (Triploid)	1	0.14	0.41	0.27	0.06	0.806
O_2 Acclimation (Normoxia)	1	0.41	0.41	5.97	1.31	0.252
Ploidy × O_2 Acclimation	1	-0.14	0.58	0.28	0.06	0.804
Residuals	212			4.53		
Interlamellar Cell Mass						

Ploidy (Triploid)	**1**	**1.21**	**0.56**	**2.75**	**26.21**	**<0.001**
O_2 Acclimation (Normoxia)	1	0.21	0.56	0.01	0.10	0.485
Ploidy × O_2 Acclimation	1	0.13	0.79	0.02	0.18	0.867
Residuals	212			0.10		
Compact Myocardium Thickness						
Ploidy (Triploid)	1	0.009	0.03	0.68	33.68	0.663
O_2 Acclimation (Normoxia)	1	0.02	0.03	0.01	0.53	0.293
Ploidy × O_2 Acclimation	1	0.0002	0.04	0.01	0.48	0.996
Residuals	212			0.02		

Table 3: Final (reduced) results of a linear mixed model testing the effects of ploidy (diploid or triploid), oxygen acclimation (normoxia or hypoxia), initial test oxygen level (average 87% or 70% air saturation), condition factor, relative ventricular mass, hepatosomatic index, interlamellar cell mass, compact myocardium thickness and hematocrit on time to loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*). Non-significant interactions and main effects (p>0.05) were removed using backward stepwise selection, with only significant main effects remaining in the final model. R^2 marginal = 0.488 and R^2 conditional = 0.772.

Source of variation	df	df residuals	Coefficient	Coefficient SE	F-value	*p*-value
Intercept			56.55	1.77		<0.001
Initial O_2	1	13.90	9.16	1.70	28.95	<0.001
Relative Ventricular Mass	1	174.30	-36.20	11.75	9.27	<0.001

Table 4: Final (reduced) results of a linear mixed model testing the effects of ploidy (diploid or triploid), oxygen acclimation (normoxia or hypoxia), initial test oxygen level (average 87% or 70% air saturation), condition factor, relative ventricular mass, hepatosomatic index, interlamellar cell mass, compact myocardium thickness and hematocrit on PO_2 at loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*). Non-significant interactions and main effects (p>0.05) were removed using backward stepwise selection, with only significant main effects remaining in the final model. R^2 marginal = 0.065 and R^2 conditional = 0.232.

Source of variation	df	df residuals	Coefficient	Coefficient SE	F-value	*p*-value
Intercept			0.01	0.04		<0.001
O_2 Acclimation	1	23.75	0.04	0.01	6.47	0.018
Ploidy	1	23.16	0.03	0.01	4.83	0.038
Hematocrit	1	174.39	0.001	0.01	4.04	0.046

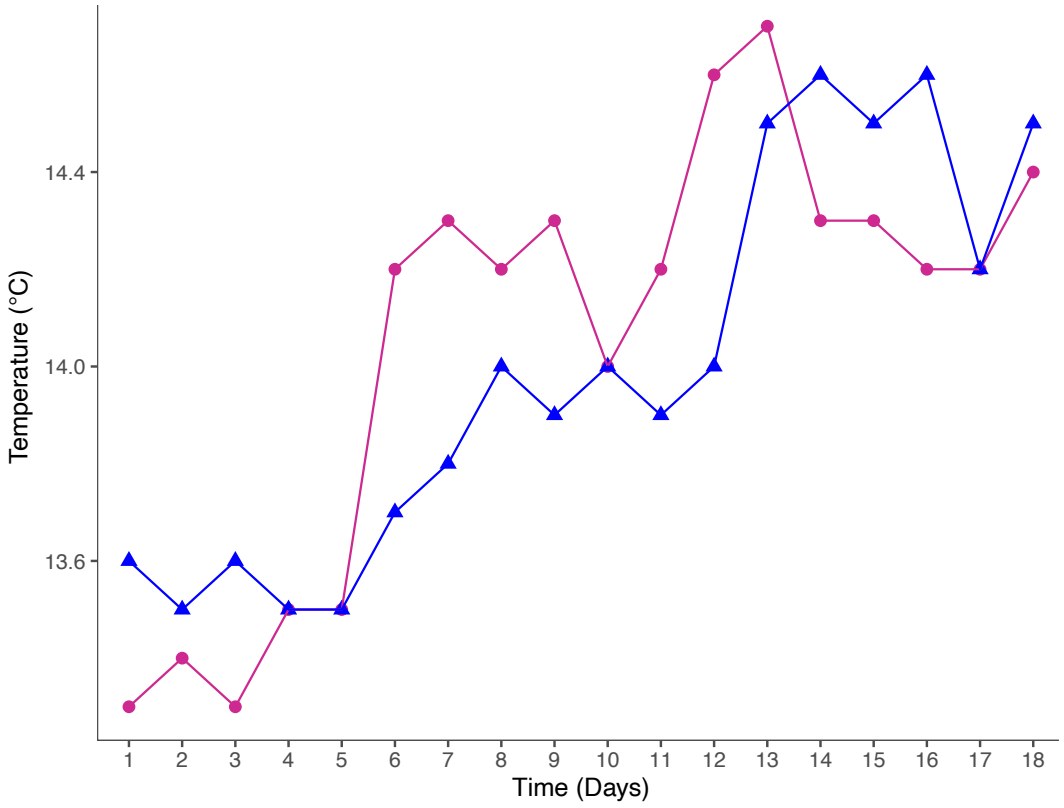

Figure 2: Average temperature of all tanks for each 18-tank system (pink circles = hypoxia tanks, blue triangles = normoxia tanks) over the course of the 18-day acute hypoxia trials. Error bars were smaller than the symbols used to plot values on the graph.

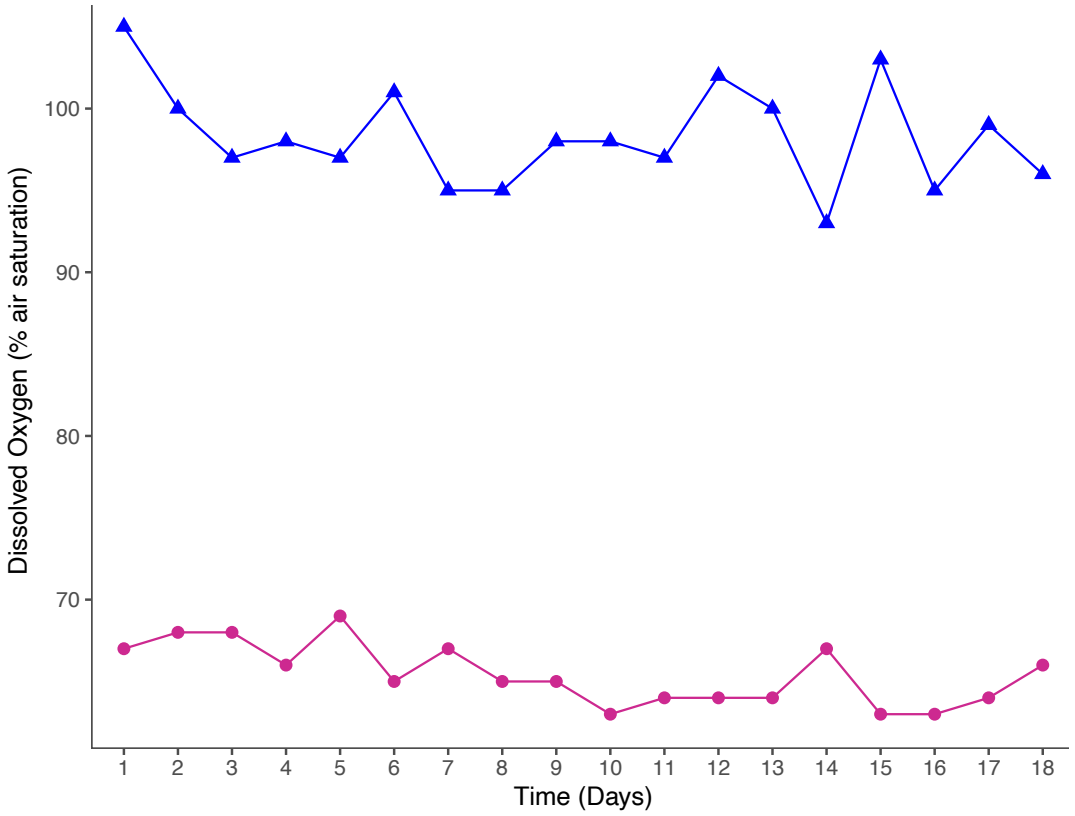

Figure 3: Average dissolved oxygen levels of all tanks for each 18-tank system (pink circles = hypoxia tanks, blue triangles = normoxia tanks) over the course of the 18-day acute hypoxia trials. Error bars were smaller than the symbols used to plot values on the graph.

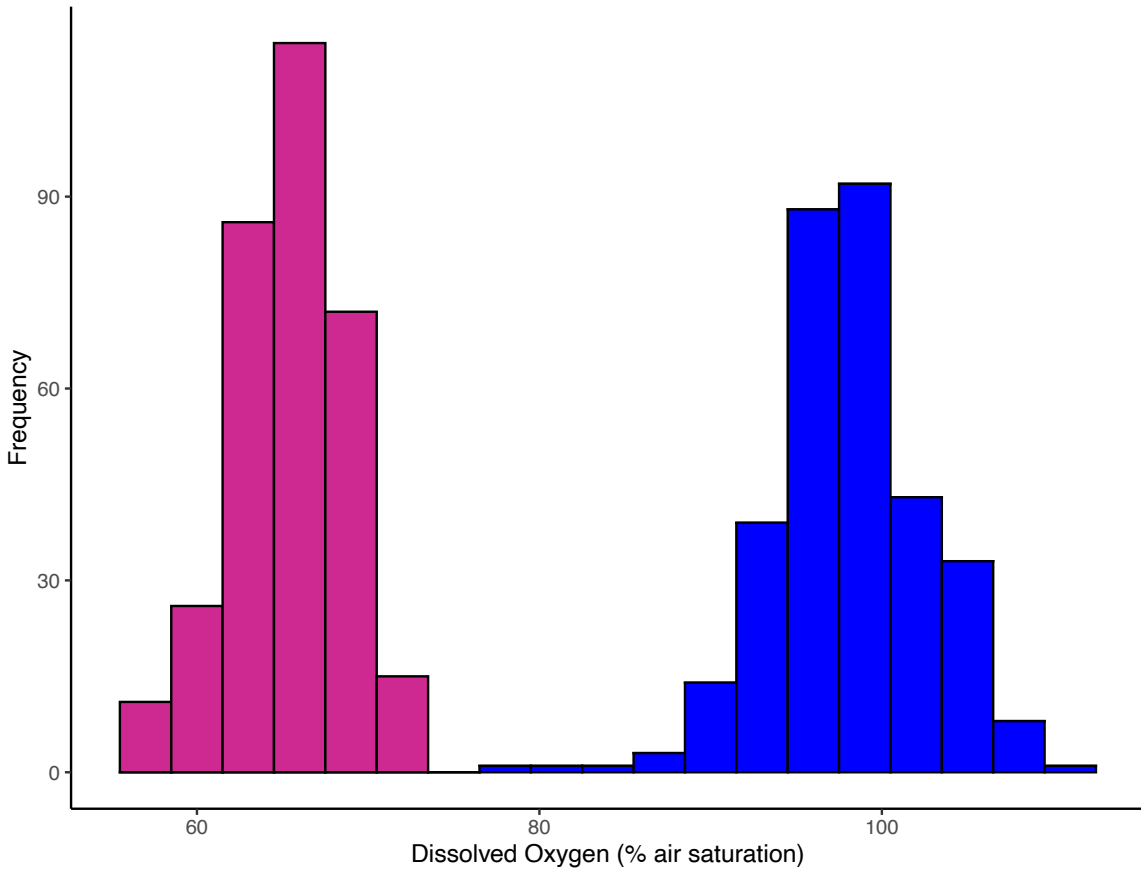

Figure 4: Average dissolved oxygen levels of all home tanks for both acclimation groups (pink = hypoxia, blue = normoxia) over the course of the 18-day acute hypoxia trials.

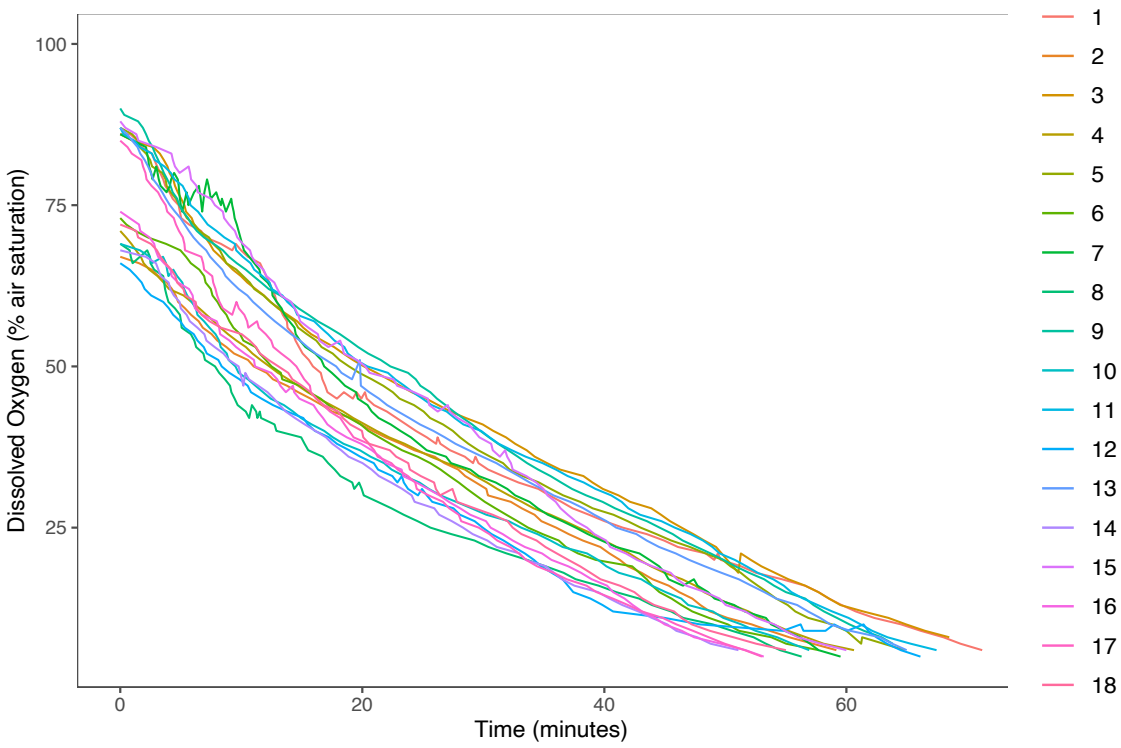

Figure 5: Rate of deoxygenation in the water bath during acute hypoxia trials (N = 18). Each line represents a single day (trial); with starting oxygen level alternating between average 87% and 70% of air saturation for 18 days.

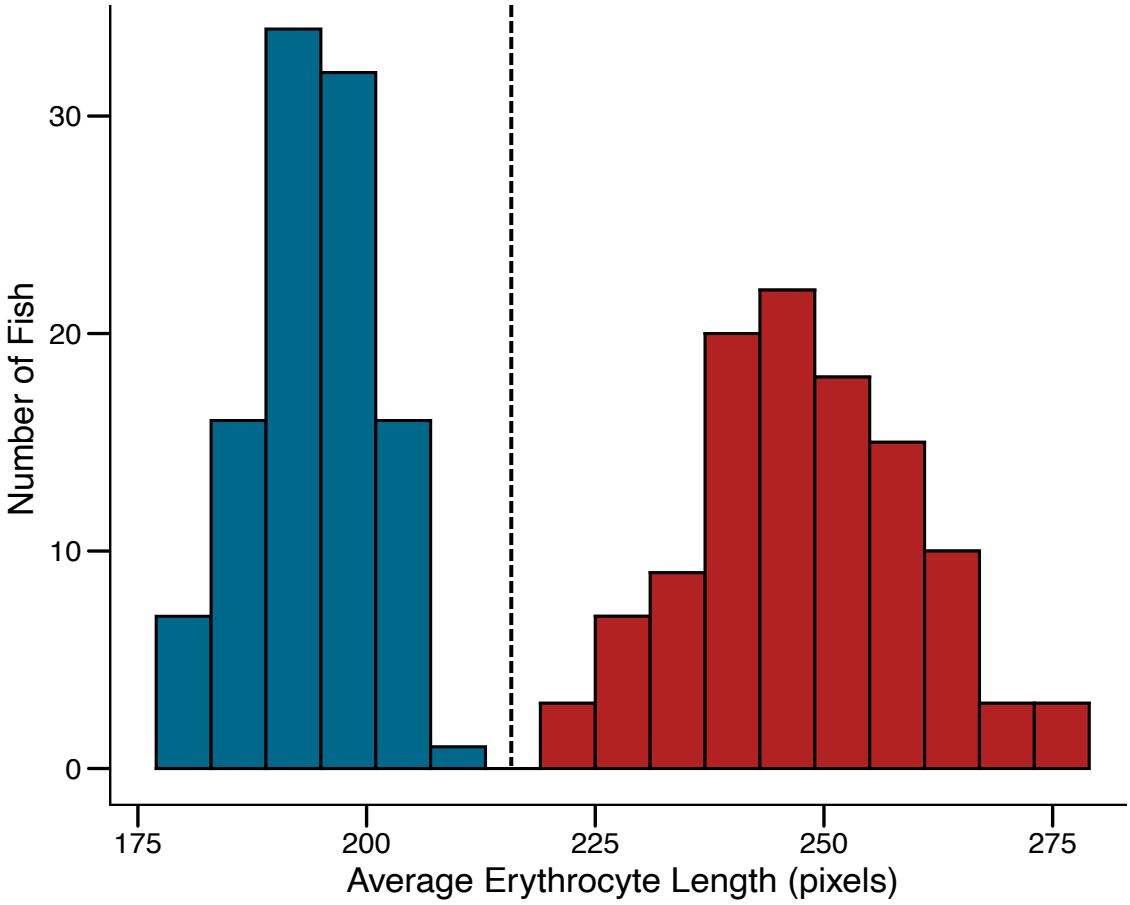

Figure 6: Average erythrocyte length of each fish that underwent the acute hypoxia trials (N = 216). The dashed line at 215 pixels was the cutoff used to classify fish as diploid (blue) or triploid (red).

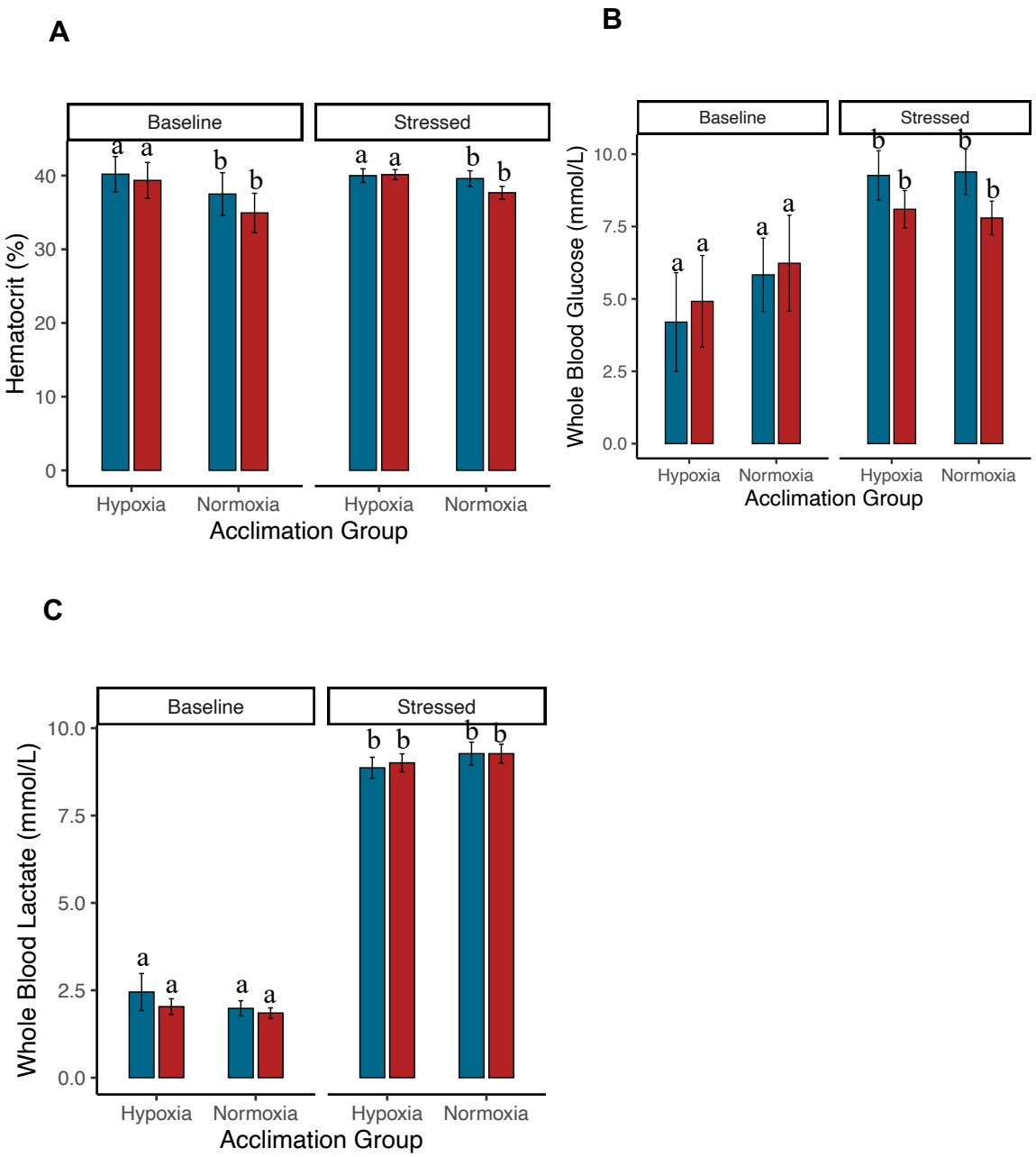

Figure 7: The effect of ploidy (diploid in blue and triploid in red), 7 weeks of acclimation to hypoxia or normoxia (65 and 98% air saturation, respectively) and treatment (baseline or stressed) on (A) hematocrit, (B) glucose, and (C) lactate levels in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; N = 240). Letters above error bars denote significant differences, taking all 8 bars into consideration as there were no significant interactions for any of the above measures (i.e., Panel A: hypoxia acclimated fish had significantly higher hematocrit compared to normoxia acclimated fish, regardless of treatment and hypoxia acclimated fish did not differ significantly from normoxia acclimated fish).

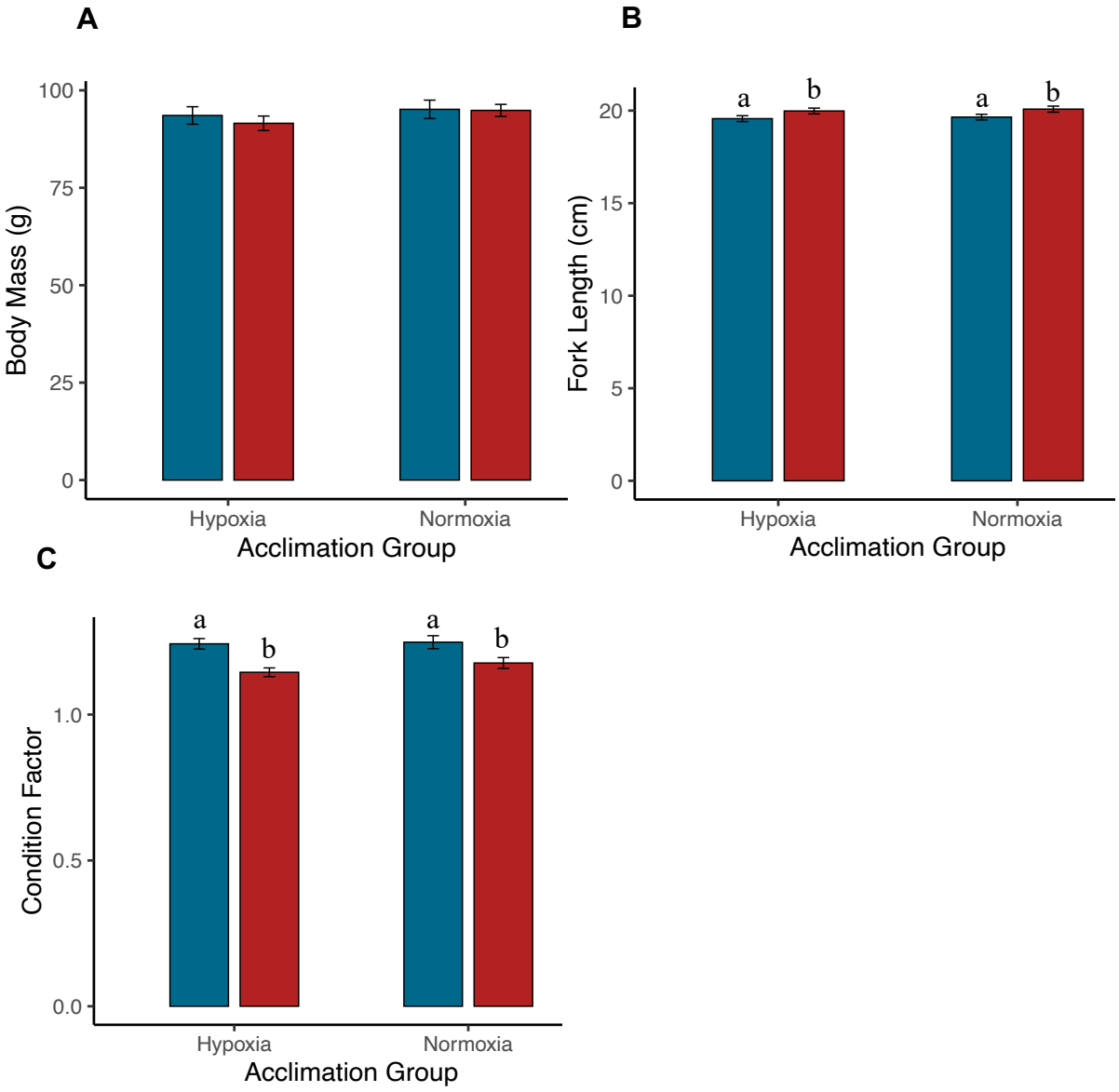

Figure 8: The effect of ploidy (diploid in blue and triploid in red) and 7 weeks of acclimation to hypoxia or normoxia (65 and 98% air saturation, respectively) on (A) body mass, (B) fork length, and (C) condition factor in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; N = 216). Letters above error bars denote significant differences.

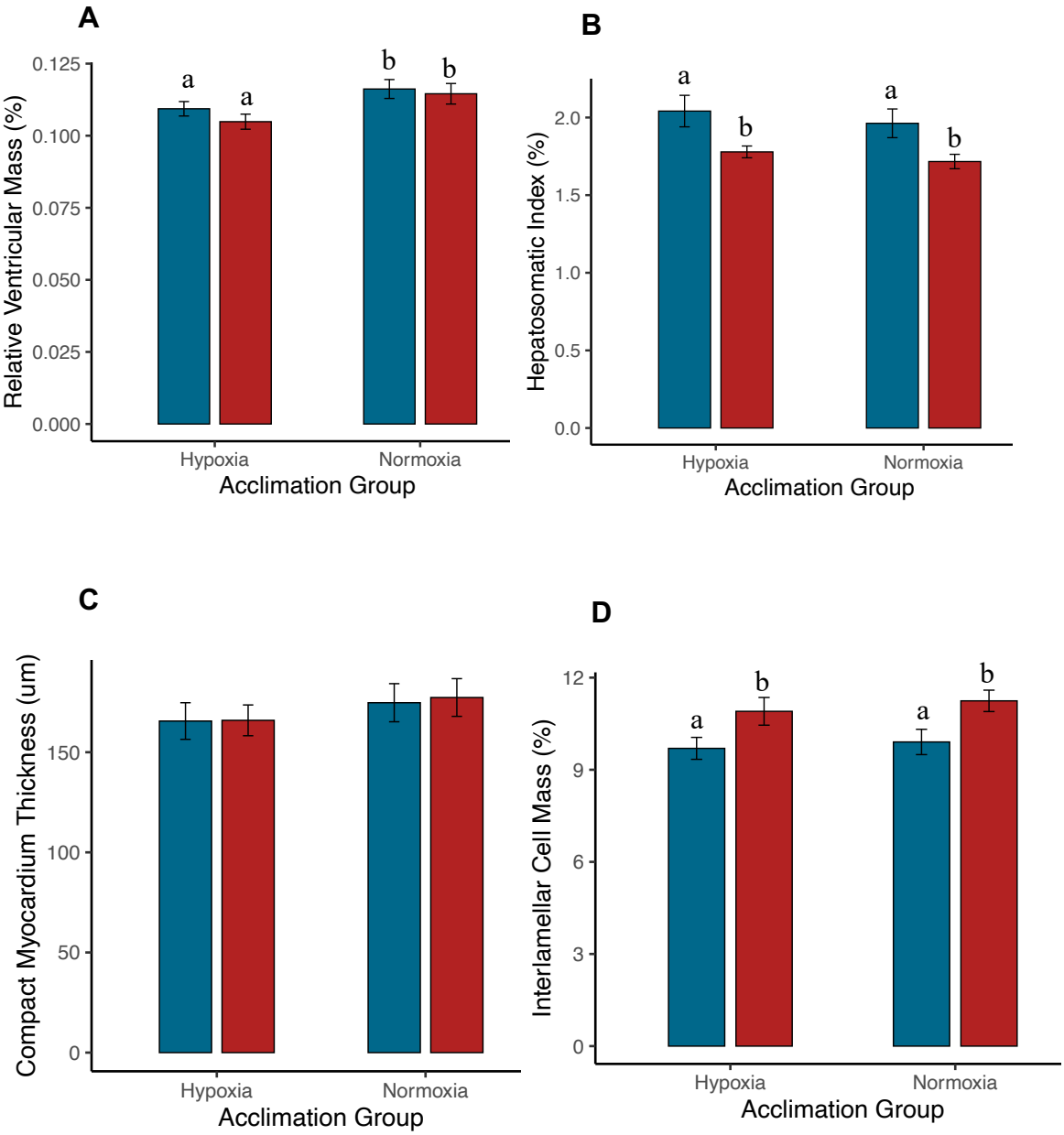

Figure 9: The effect of ploidy (diploid in blue and triploid in red) and 7 weeks of acclimation to hypoxia or normoxia (65 and 98% air saturation, respectively) on (A) relative ventricular mass, (B) hepatosomatic index, (C) compact myocardium, and (D) interlamellar cell mass in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; N = 216). Letters above error bars denote significant differences.

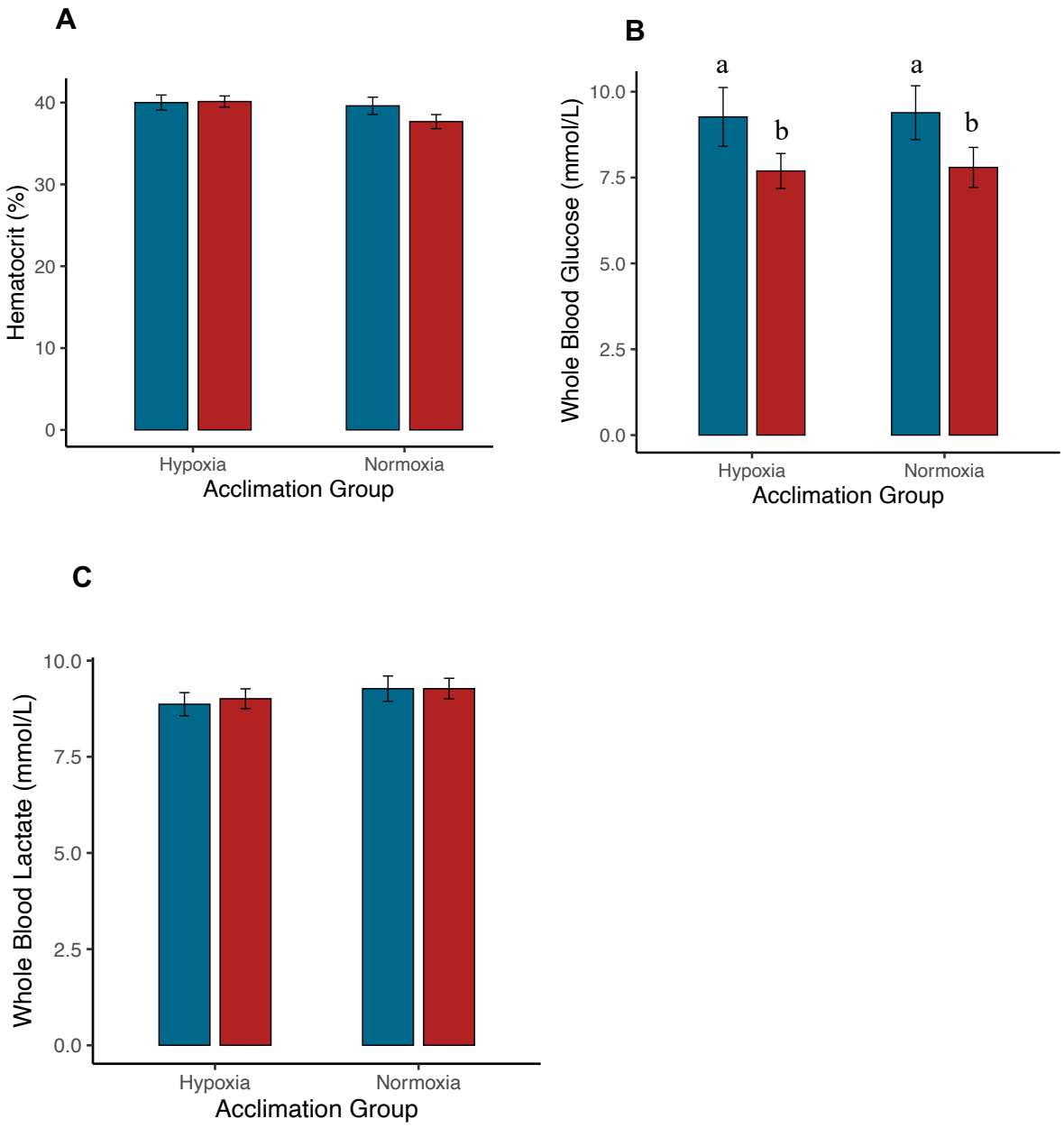

Figure 10: The effect of ploidy (diploid in blue and triploid in red) and 7 weeks of acclimation to hypoxia or normoxia (65 and 98% air saturation, respectively) on (A) hematocrit, (B) glucose, and (C) lactate levels in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (mean ± SE; N = 216). Letters above error bars denote significant differences.

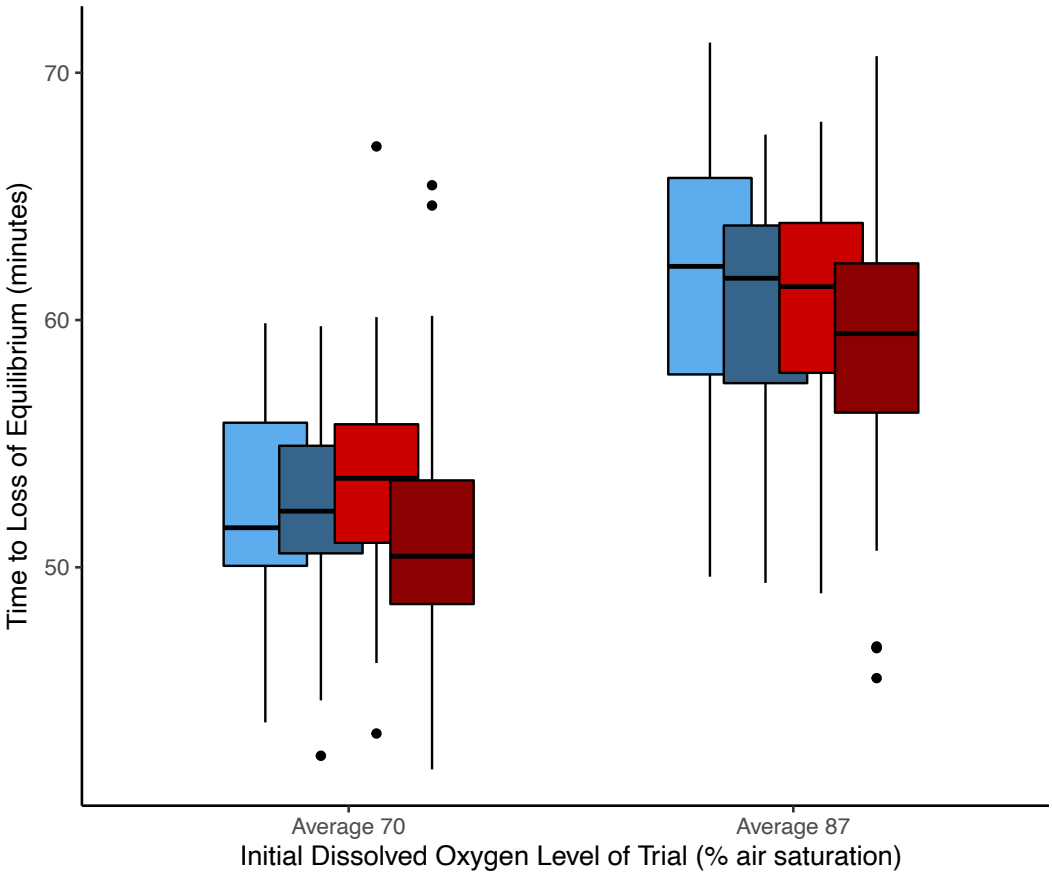

Figure 11: The effect of ploidy (diploid in blue and triploid in red), oxygen acclimation (hypoxia light coloured and normoxia dark coloured), and daily oxygen starting level of the acute hypoxia trials (average 87% or 70% of air saturation) on time to loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (N = 216). Box represents the 25–75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

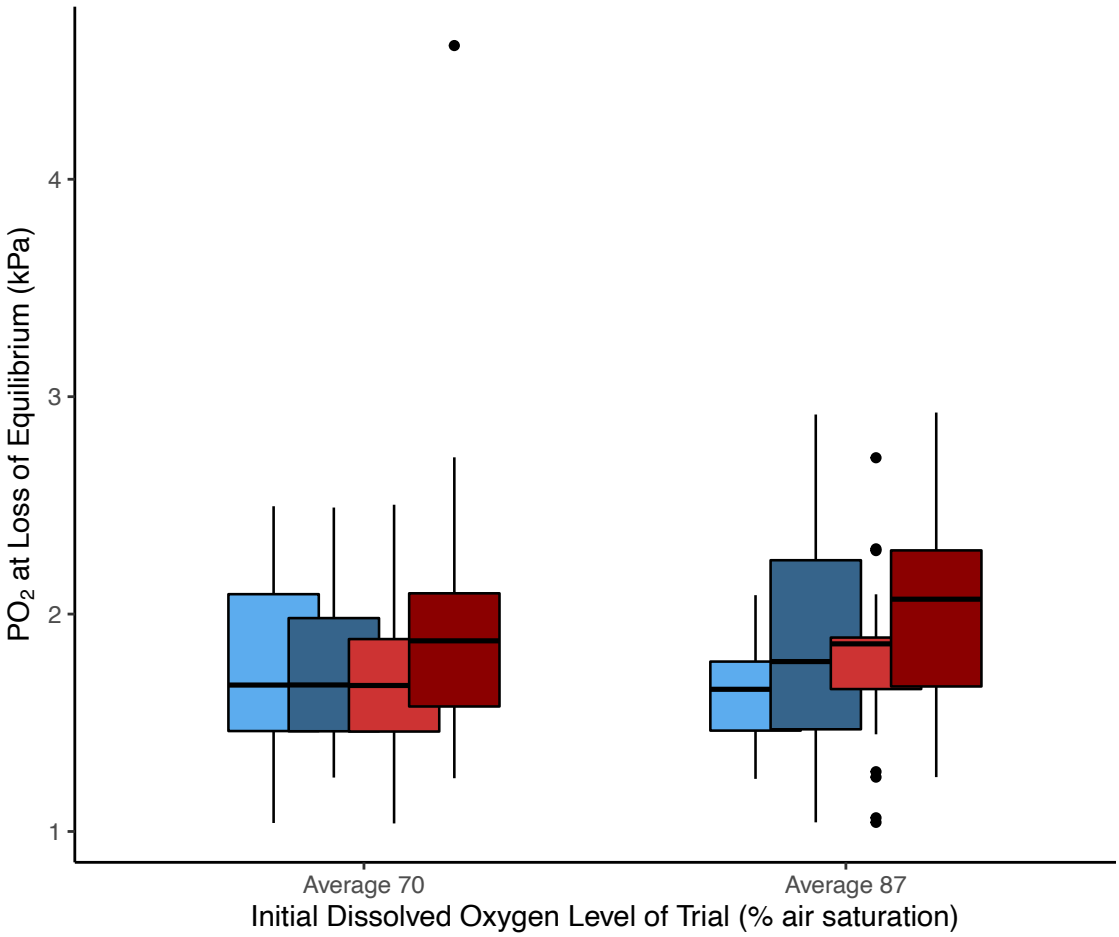

Figure 12: The effect of ploidy (diploid in blue and triploid in red), oxygen acclimation (hypoxia light coloured and normoxia dark coloured), and daily oxygen starting level of the acute hypoxia trials (average 87% or 70% of air saturation) on PO_2 at loss of equilibrium in juvenile brook charr (*Salvelinus fontinalis*) subjected to hypoxia challenges (N = 216). Box represents the 25–75% quantiles, midline represents the median, and dots represent outliers more than 1.5 interquartile ranges from the nearest quartile.

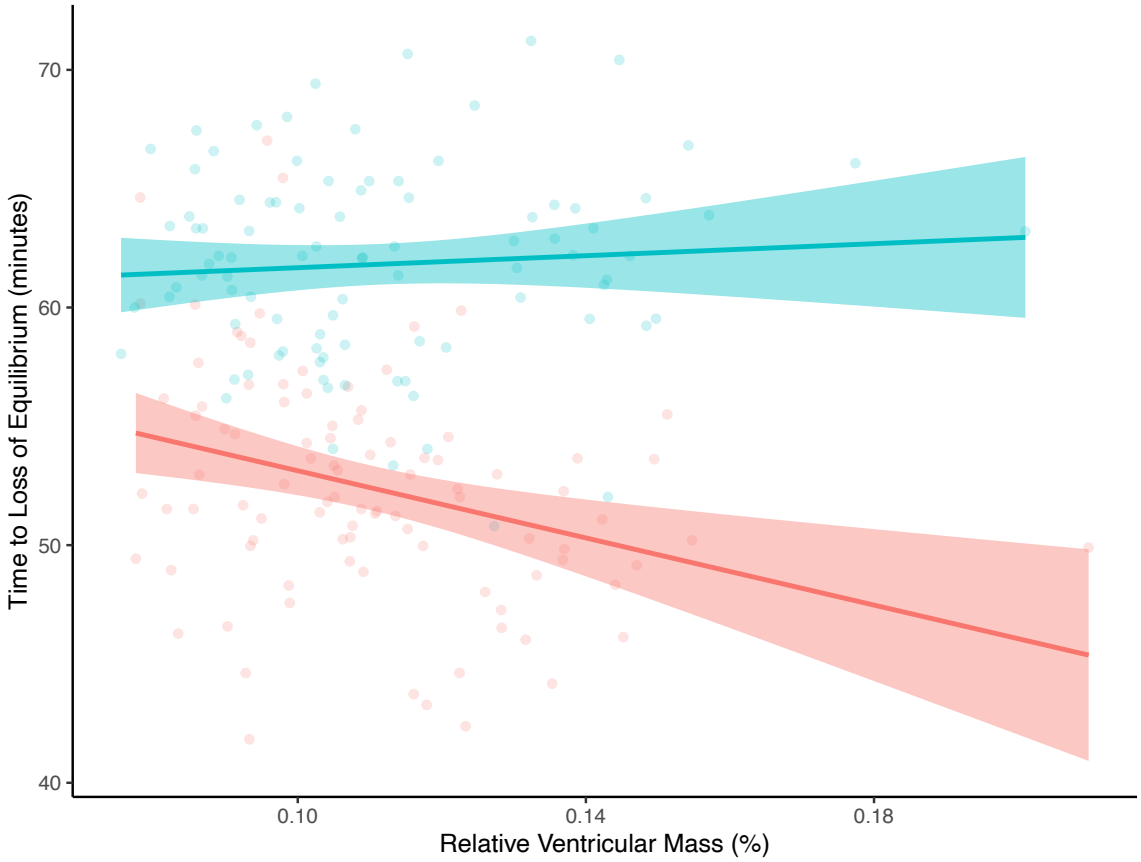

Figure 13: The effect of relative ventricular mass on time to loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to an acute hypoxia challenge starting at two different oxygen levels: average 87% (blue) and 70% (pink) air saturation (N = 188). Solid line represents linear regression of combined diploids and triploids, coloured area is 95% confidence interval**.**

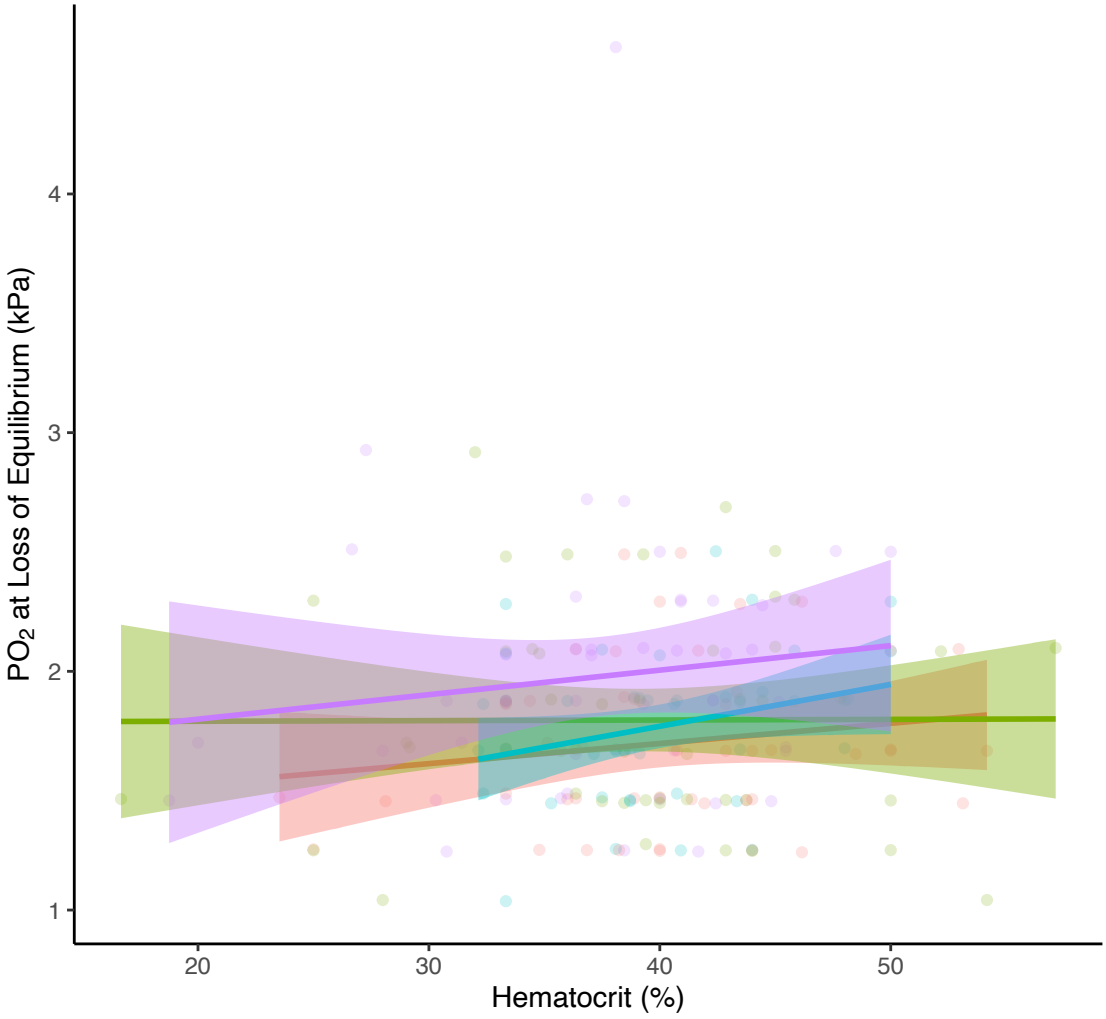

Figure 14: The effect hematocrit on PO2 at loss of equilibrium in diploid and triploid juvenile brook charr (*Salvelinus fontinalis*) subjected to an acute hypoxia challenge starting at two different oxygen levels: (average 87% and 70% air saturation); 2N70 (pink), 3N70 (blue), 2N100 (green), 3N100 (purple) (N = 188). Solid line represents linear regression, coloured area is 95% confidence interval.

4. DISCUSSION

The main objective of this study was to determine if acclimation to chronic moderate hypoxia improves acute hypoxia tolerance of triploid fish. If improved hypoxia tolerance is evident, these findings could potentially be useful in a commercial setting, as the use of sterile triploids could help mitigate genetic introgression caused by farmed escapees (Benfey, 2016). Additionally, it is beneficial to know more about how fish respond to chronic hypoxia, as instances of hypoxia commonly occur in aquatic environments and are becoming more severe and frequent due to climate change (Corkum and Gamperl, 2009; Vaquer-Sunyer and Duarte, 2011; Stehfest et al., 2017; Oldham et al., 2019; Brauner and Richards, 2020; Strowbridge et al., 2021). Taken together, results from this study support an overall trend that hypoxia acclimation improved acute hypoxia tolerance for all fish, but triploids were less hypoxia tolerant than diploids. Since hypoxia-acclimated triploids (3N70) outperformed normoxia-acclimated diploids (2N100), an argument can be made for increasing acute hypoxia tolerance in triploids by way of acclimation to moderate hypoxia.

Fish acclimated to hypoxia (65% air saturation) did show an improvement in hypoxia tolerance via a lower oxygen tension (PO_2) at LOE. Although this did not translate to a significantly longer time to LOE, it was trending toward significance (*p*-value = 0.09; Appendix II). Very few studies have looked at using chronic hypoxia acclimation (longer than 7 days) to test hypoxia tolerance in fish.; however, one of the few that did (Dan et al., 2014) also found that hypoxia acclimation improved hypoxia tolerance, based on P_{crit} and PO_2 at LOE. As previously stated, both P_{crit} and PO_2 at LOE

are the two of the recommended ways to quantify hypoxia tolerance (Brauner and Richards, 2020), with LOE tests suggested to be a better measure of hypoxia tolerance (Wood, 2018).

Triploids had a higher PO_2 at LOE compared to diploids, indicating triploids had a lower hypoxia tolerance. This supports past research which consistently shows that diploids perform better than triploids under hypoxic conditions (Hansen et al., 2015; Scott et al., 2015; Sambraus et al., 2017; Benfey and Devlin, 2018; Jensen and Benfey, 2022). This effect of ploidy was not seen on time to LOE; however, a non-significant trend that diploids had a longer time to LOE than triploids was observed. There has been much speculation as to why triploids consistently show reduce hypoxia tolerance. One suggestion is that due to the reduced cell surface area to volume ratio of triploids, gas exchange rates may be reduced, lowering their ability to transport oxygen, causing a decrease in cellular respiration (Small and Benfey, 1987; Benfey, 1999). Another suggestion is that triploids have a reduced aerobic scope (Altimiras et al., 2002; Rodrigues et al., 2020) or increased standard metabolic rate (O'Donnell et al., 2017), but contradicting results have been found in the literature. Some studies found little to support differences in aerobic scope of diploid and triploid Atlantic salmon (Lijalad and Powell, 2009; Bowden et al., 2018) and the SMR of diploid and triploid brook charr (Daigle et al., 2021). In contrast, Hyndman et al., (2003b) found triploid brook charr had the same routine metabolic rate as diploids, but a lower MMR following exhaustive exercise at 16°C, indicating a reduced aerobic scope, and Leal et al. (2021) found indications of reduced energy availability for processes above basal maintenance in

triploid white sturgeon (*Acipenser transmontanus*). It is also possible that a larger ploidy effect would be seen if the acute hypoxia trials were done at a warmer temperature. Benfey and Devlin (2018) found a significant reduction in triploid hypoxia tolerance relative to diploids at temperatures above 13°C, with differences between ploidies increasing with greater temperatures. The initial dissolved oxygen level of the trials in my experiments did not affect PO_2 at LOE, but fish that started the acute hypoxia trials at 70% air saturation reached LOE sooner because they started at a dissolved oxygen closer to that at LOE. Triploids also had a longer fork length (overall model was not significant, it was approaching significance; *p*-value = 0.060) and, therefore, a lower condition factor compared to diploids, which is consistent with previous findings (Ojolick et al., 1995; Fjelldal and Hansen, 2010; Hansen et al., 2015; Sambraus et al., 2017; Peruzzi et al., 2018; Sambraus et al., 2018; Jensen and Benfey, 2022).

Hematocrit had a significant effect on hypoxia tolerance, with higher hematocrit associated with a higher PO_2 at LOE (i.e., reduced hypoxia tolerance). This contradicts Jensen and Benfey (2022), where higher hematocrit was associated with a longer time to reach LOE (i.e., increased hypoxia tolerance). Several other studies show a positive relationship between hematocrit and presumed hypoxia tolerance, with fish acclimated to hypoxia having increased hematocrit (Remen et al., 2012; Sambraus et al., 2017; Sambraus et al., 2018; Gamperl et al., 2020). When looking only at fish that underwent the acute hypoxia trials in my study (n=216), there was no effect of hypoxia acclimation on hematocrit, regardless of ploidy. However, when combined with those that did not undergo the acute hypoxia trials (n=24), fish acclimated to hypoxia had significantly

higher hematocrit, suggesting improved hypoxia tolerance and aligning with these other findings. Hematocrit is an inexpensive and quick way to estimate the oxygen carrying capacity of the blood; however, measuring blood hemoglobin concentration in this study could have provided a more detailed estimate on oxygen carrying capacity of the blood.

There was no significant effect of ploidy or hypoxia acclimation on resting (baseline) glucose and lactate concentrations, which is supported by others (Hyndman et al., 2003a; Fu et al., 2011). Like previous findings, there was also no difference in baseline hematocrit between ploidies (Small and Benfey, 1987; Altimiras et al., 2002; Cogswell et al., 2002); however, as mentioned above, fish acclimated to hypoxia had higher baseline hematocrit compared to normoxia acclimated fish. When comparing baseline (un-stressed) fish with fish that underwent the acute hypoxia trials, both glucose and lactate concentrations were elevated in fish that underwent the acute hypoxia trials, regardless of ploidy. This glucose increase is likely due to the elevated catecholamine levels in response to the stressor, in turn increasing blood glucose levels (Pottinger, 1998), but should also consider that the overall model for glucose was not significant, although it was approaching significance (p-value = 0.068). The increased lactate is likely due to glycogen stores being depleted and lactate accumulating in the muscle tissues before being released into the blood (Milligan and Girard, 1993).

Looking solely at stressed fish (i.e., those that underwent acute hypoxia trials; Figure 10), triploids had lower glucose concentrations compared to diploids, but there was no significant difference in lactate between ploidies. Other studies have found lower glucose levels in triploids than in diploids following a stressor (Hansen et al., 2015;

Sambraus et al., 2017; Sambraus et al., 2018), with Sambraus et al. (2018) noting that at colder temperatures triploid Atlantic salmon successfully maintain muscle glycogen, and they actually perform better than diploids. The current study was conducted at 13.5°C, which is at the lower end of temperatures for hypoxia studies, and perhaps this is why triploids had lower glucose levels compared to diploids, although the overall model for glucose was not significant (p-value = 0.159). Hansen et al. (2015) also found no difference in lactate concentrations between ploidies. There has been some speculation as to what oxygen level is hypoxic enough to see some of these differences, as Sambraus et al. (2017) only found increases in lactate concentrations in fish acclimated to 50% air saturation or lower.

Relative ventricular mass (RVM) had a significant effect on hypoxia tolerance, with an increase in RVM associated with a shorter time to LOE (i.e., decreased hypoxia tolerance). Additionally, hypoxia-acclimated fish had a lower RVM than normoxia-acclimated fish. This is contrary to the expectation that fish with a higher RVM should have a better hypoxia tolerance, since RVM predicts cardiac output, especially in response to stressors (Farrell et al., 2009). Although a decreased RVM in hypoxia-acclimated fish was an unexpected result, it is consistent with studies showing a decreased RVM in response to warm-acclimation in salmonids (Anttila et al., 2015; Jensen and Benfey, 2022), with Jensen and Benfey further documenting a return to higher RVM when reacclimated to colder temperatures. This aligns with a study done by Klaiman et al. (2011) that found cold-acclimated rainbow trout had a significantly greater RVM compared to the control group and they suggested that cellular hypertrophy is the

main driving force for increased RVM in response to cold acclimation. More recent work by Roberts (2016) found a hypoxia-induced decline in ventricular output in rainbow trout, so perhaps the hypoxia-acclimated fish in this study responded (via decreased RVM) to the environmental stressor of low oxygen similarly. There is also the possibility that hypoxia acclimation alone is not enough to alter RVM (Farrell et al., 2009), with Gamperl et al. (2020) finding that the combination of warm temperature with hypoxia was needed to see an increase in RVM. There was no effect of ploidy on RVM in this study. Past research on Atlantic salmon has found either no effect of triploidy (Fraser et al., 2013; Bowden et al., 2018) or significantly larger RVM in triploids (Fraser et al., 2015), but most studies have focused more on thermal acclimation than any other stressor, indicating a need to further explore how hypoxia acclimation can affect RVM.

Since ventricular mass consists of both compact and spongy myocardium, they are often measured alongside one another, with an increase in RVM correlating with increase in compact and/or spongy myocardium thickness (Klaiman et al., 2011) and improving hypoxia tolerance. In this study, only compact myocardium thickness was measured, as it requires less time than computing a spongy:compact myocardium ratio. Additionally, since spongy myocardium is consistently exposed to low oxygen (Imbrogno et al., 2022), it has been shown that it does not benefit (and remodel) in response to hypoxia acclimation in the same way compact myocardium does (Roberts et al., 2021). In fact, Carnevale et al. (2020) found that hypoxia acclimation negatively impacted the contractility of the spongy myocardium in steelhead trout (*Oncorhynchus mykiss*). There was no significant effect of compact myocardium thickness on hypoxia

tolerance in this study, potentially because hypoxia alone was not enough to trigger compact myocardial growth (Farrell et al., 2009). Both hypoxia and normoxia-acclimated fish were reared at 13.5°C, and perhaps higher temperatures in combination hypoxia are needed to see the full extent of cardiac remodelling.

Hepatosomatic index (HSI) has been used as a predictor of hypoxia tolerance and is generally a good indication of energy stores in fish. A decreased HSI is associated with decreased energy reserves, indicating that energy is being put elsewhere (e.g., to hypoxia response) or that there is reduced energy uptake (De Boeck et al., 2013). Hypoxia acclimation did not affect HSI and there was no effect of HSI on hypoxia tolerance in my study, but this could be because hypoxia alone may not always be enough to trigger changes in HSI. De Boeck et al. (2013) only saw effects of hypoxia on HSI when combined with fasting. Triploids had a lower HSI than diploids, which supports some studies (Johnson et al., 1986; Felip et al., 2001), but others have found triploids having higher HSI (Cantas et al., 2011, Kizak et al., 2013). One theory as to why triploids would have a smaller HSI than diploids is that they may be diverting more energy to maintenance rather than to growth (O'Donnell et al., 2017). This is consistent with a lower condition factor in triploids, which is also an indication of reduced energy stores (Benfey, 2016).

Interlamellar cell mass (ILCM) was significantly greater in triploids compared to diploids. This would indicate a reduced functional gill surface area for respiratory gas exchange with the water in triploids, and therefore potentially reduced hypoxia tolerance, consistent with findings from Sadler et al. (2001). However, it should be noted that

Sadler et al. (2001) used photographic contact prints to measure overall gill surface area at a macroscopic scale whereas I measured lamellar length and size of the ILCM, an approach similar to that of Ong et al. (2007), to gain information on functional gill surface area. Another reason why percentage of ILCM space filled with cells was used for this specific study is that past research found diploids had significantly shorter lamellae compared to triploids (Flajšhans and Piačková, 2006).

There was no effect of hypoxia acclimation on ILCM, which is surprising. Past research has shown a reduction in ILCM in crucian carp after just one day of hypoxia exposure (Sollid et al., 2003). In fact, they saw no further changes in gill morphology after 7 days of hypoxia exposure, suggesting that a week should be more than long enough to see any changes in gill morphology. So, it is very unexpected to not see any distinct hypoxia-acclimated induced gill morphology in fish acclimated to hypoxia for 7 weeks. There has also been research showing that hypoxia, coupled with increased water temperature results in the most drastic gill remodelling (Sollid et al., 2005). Additionally, there are up to six ways fish can change functional gill surface area and diffusion distance, either through reorganization of blood flow pathways or through reversible gill remodelling (Wood and Eom, 2021). Gill remodelling can be achieved through increased ILCM size, proliferation of ionocytes up the sides of the lamellae and extending pavement cells to cover the apical exposure of ionocytes. Sadler et al. (2001) used the approach of measuring gill surface area, whereas for my study, ILCM size was measured. Perhaps, to gain a more complete understanding of gill remodelling, it would be

beneficial to explore some of these additional ways fish can change functional gill surface area in response to hypoxia.

4.1 Conclusions and Future Research

Aside from acute hypoxia tolerance (PO_2 at LOE) and RVM, there were no effects of hypoxia acclimation on the parameters measured in fish that completed the acute hypoxia trials. However, when combining the fish that underwent the acute hypoxia trials with un-stressed fish, hypoxia-acclimated fish also had significantly higher hematocrit compared to normoxia-acclimated fish. Effects of ploidy were observed in fork length (overall model not significant but approaching significance; p-value = 0.068), condition factor, HSI, glucose concentrations (overall model not significant but approaching significance; p-value = 0.060), ILCM and PO_2 at LOE. When combined with additional (non-significant) trends observed in this study, a picture emerges of performance during the acute hypoxia trials (best to worst) as: 2N70 > 3N70 > 2N100 > 3N100, as predicted. This indicates that triploid performance was better than diploids when comparing hypoxia-acclimated triploids to normoxia-acclimated diploids. This could be an indication that at higher temperatures, or perhaps with a larger-scale experiment, hypoxia-acclimation could improve triploid performance in sub-optimal conditions.

Some considerations for future studies would be to lower the percentage of air saturation for hypoxia acclimation. Others have shown that lowering hypoxia levels to 60, 50 or 40% air saturation increases the physiological response to hypoxia in many fish species (Burt et al., 2012; Remen et al., 2012; Sambraus et al., 2017). It could also be

beneficial to combine hypoxia with other stressors (i.e., fasting, higher temperature), as it is not only more environmentally relevant, but has been shown to induce a greater physiological response. This study was designed to look at hypoxia-acclimation and triploidy, and it has shown the capacity for hypoxia acclimation to improve triploid performance and certainly warrants further investigation.

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Appendix I

Hematoxylin and Eosin Staining Procedure

Toluene 1	5 minutes	De-wax
Toluene 2	5 minutes	De-wax
100% Ethanol	2 minutes	
100% Ethanol	2 minutes	
95% Ethanol	2 minutes	
70% Ethanol	2 minutes	
50% Ethanol	2 minutes	
Dist. Water	2 minutes	
Hematoxylin Gill III *		2 minutes
Dist. Water	Rinse	(Carry to sink in square dish of water)
Tap Water	15 minutes	(Running slowly – cold only)
70% Ethanol	2 minutes	
100% Ethanol	2 minutes	Agitate - discard to waste bottle after use
100% Ethanol	2 minutes	Transfer to 100% Ethanol 1 after use
Toluene 1	2 minutes	
Toluene 2	2 minutes **	

Coverslip with DPX in the Fume Hood. Wear Nitrile gloves.
Slides should be kept in 37°C oven overnight for the DPX to solidify.

*Hematoxylin needs to be filtered before use regularly.

Plus, time to coverslip slides. Slides **must be kept in Toluene during cover slipping so they **never dry out**.

Masson's Trichome Staining Procedure

Toluene 1	5 minutes	De-wax
Toluene 2	5 minutes	De-wax
100% Ethanol	2 minutes	
100% Ethanol	2 minutes	
95% Ethanol	2 minutes	
70% Ethanol	2 minutes	
50% Ethanol	2 minutes	
Dist. Water	2 minutes	

Hematoxylin Gill III *		1 min 20 sec
Dist. Water	Rinse	(Carry to sink in square dish of water)
Tap Water	15 minutes	(Running slowly – cold only)
Dist. Water	Rinse	1 minute

Stain in Ponceau Acid Fuchsin 2 minutes
Rinse in distilled water

a. **Use three pots of distilled water** for best rinse and less contamination of next solution (10 sec 1st pot, 20 sec 2nd pot, 30 sec 3rd pot). Agitate.

Differentiate in 1% Aqueous Phosphomolybdic Acid 5 minutes
DO NOT RINSE. Transfer to Acetic Aniline Blue 3 minutes
Rinse in distilled water

a. **Use three pots of distilled water** for best rinse and less contamination of next solution (10 sec 1st pot, 20 sec 2nd pot, 30 sec 3rd pot). Agitate.

1% Aqueous Phosphomolybdic Acid	5 minutes
Place in 1% glacial acetic acid	3 minutes
Dehydrate in 95% Ethanol	2 minutes
100% Ethanol	2 minutes
100% Ethanol	2 minutes
Toluene 1	2 minutes
Toluene 2	2 minutes **

Coverslip with DPX in the Fume Hood. Wear Nitrile gloves.
Slides should be kept in 37°C oven overnight for the DPX to solidify.

*Hematoxylin needs to be filtered before use regularly.

Plus, time to coverslip slides. Slides **must be kept in Toluene during cover slipping so they **never dry out.**

Appendix II

Time to loss of equilibrium backward stepwise selection

Step 1 (All Terms Included):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.31	0.082
Ploidy	1	0.93	0.343
Initial O_2	1	31.17	<0.001
Condition Factor	1	2.08	0.152
Relative Ventricular Mass	1	3.80	0.053
Hepatosomatic Index	1	0.09	0.762
Compact Myocardium Thickness	1	0.25	0.620
Interlamellar Cell Mass	1	0.26	0.614
Hematocrit	1	1.82	0.180
O_2 Acclimation × Ploidy	1	0.38	0.544
O_2 Acclimation × Initial O_2	1	0.76	0.387
Ploidy × Initial O_2	1	1.22	0.272
Acclimation × Ploidy × Initial O_2	1	1.24	0.268

Step 2 (Removed Hepatosomatic Index):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.32	0.082
Ploidy	1	1.13	0.299
Initial O_2	1	31.15	<0.001
Condition Factor	1	2.22	0.138
Relative Ventricular Mass	1	4.29	0.040
Compact Myocardium Thickness	1	0.27	0.604
Interlamellar Cell Mass	1	0.28	0.597
Hematocrit	1	2.07	0.152
O_2 Acclimation × Ploidy	1	0.38	0.543
O_2 Acclimation × Initial O_2	1	0.80	0.374
Ploidy × Initial O_2	1	1.18	0.280
Acclimation × Ploidy × Initial O_2	1	1.25	0.267

Step 3 (Removed Compact Myocardium Thickness):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.46	0.076
Ploidy	1	1.12	0.300
Initial O_2	1	30.65	<0.001
Condition Factor	1	2.35	0.127
Relative Ventricular Mass	1	4.37	0.038
Interlamellar Cell Mass	1	0.21	0.647
Hematocrit	1	2.28	0.133
O_2 Acclimation × Ploidy	1	0.38	0.547
O_2 Acclimation × Initial O_2	1	0.86	0.355
Ploidy × Initial O_2	1	1.13	0.289
Acclimation × Ploidy × Initial O_2	1	1.31	0.255

Step 4 (Removed Interlamellar Cell Mass):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.43	0.077
Ploidy	1	1.02	0.322
Initial O_2	1	30.32	<0.001
Condition Factor	1	2.54	0.113
Relative Ventricular Mass	1	4.57	0.034
Hematocrit	1	2.40	0.123
O_2 Acclimation × Ploidy	1	0.36	0.553
O_2 Acclimation × Initial O_2	1	0.82	0.367
Ploidy × Initial O_2	1	1.08	0.302
Acclimation Ploidy × Initial O_2	1	1.36	0.247

Step 5 (Removed O_2 Acclimation × Ploidy × Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.33	0.081
Ploidy	1	1.01	0.326
Initial O_2	1	29.68	<0.001
Condition Factor	1	2.73	0.100
Relative Ventricular Mass	1	4.55	0.034
Hematocrit	1	2.21	0.139
O_2 Acclimation × Ploidy	1	0.35	0.561
O_2 Acclimation × Initial O_2	1	0.86	0.357
Ploidy × Initial O_2	1	1.04	0.309

Step 6 (Removed O_2 Acclimation × Ploidy):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.43	0.076
Ploidy	1	1.03	0.320
Initial O_2	1	29.77	<0.001
Condition Factor	1	2.92	0.089
Relative Ventricular Mass	1	4.65	0.033
Hematocrit	1	2.14	0.146
O_2 Acclimation × Initial O_2	1	0.80	0.374
Ploidy × Initial O_2	1	1.11	0.294

Step 7 (Removed O_2 Acclimation × Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.65	0.067
Ploidy	1	1.10	0.304
Initial O_2	1	29.96	<0.001
Condition Factor	1	2.79	0.097
Relative Ventricular Mass	1	5.57	0.019
Hematocrit	1	2.05	0.154
Ploidy × Initial O_2	1	1.36	0.246

Step 7 (Removed Ploidy × Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.69	0.066
Ploidy	1	1.07	0.311
Initial O_2	1	29.52	<0.001
Condition Factor	1	2.32	0.130
Relative Ventricular Mass	1	5.13	0.024
Hematocrit	1	2.21	0.139

Step 8 (Removed Ploidy):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.80	0.062
Initial O_2	1	29.87	<0.001
Condition Factor	1	1.72	0.192
Relative Ventricular Mass	1	5.14	0.025
Hematocrit	1	2.03	0.156

Step 9 (Removed Condition Factor):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.91	0.058
Initial O_2	1	29.98	<0.001
Relative Ventricular Mass	1	5.16	0.024
Hematocrit	1	1.94	0.165

Step 10 (Removed Hematocrit):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.11	0.090
Initial O_2	1	29.70	<0.001
Relative Ventricular Mass	1	8.11	<0.001

Step 11 (Removed O_2 Acclimation):

Source of variation	df	F-value	*p*-value
Initial O_2	1	28.95	<0.001
Relative Ventricular Mass	1	9.27	<0.001

PO_2 at loss of equilibrium using backward stepwise selection

Step 1 (All Terms Included):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	3.99	0.058
Ploidy	1	3.31	0.080
Initial O_2	1	0.04	0.854
Condition Factor	1	0.80	0.372
Relative Ventricular Mass	1	1.22	0.271
Hepatosomatic Index	1	1.32	0.252
Compact Myocardium Thickness	1	0.08	0.779
Interlamellar Cell Mass	1	0.20	0.658
Hematocrit	1	1.75	0.188
O_2 Acclimation × Ploidy	1	0.70	0.414
O_2 Acclimation × Initial O_2	1	0.96	0.331
Ploidy × Initial O_2	1	0.63	0.431
Acclimation × Ploidy × Initial O_2	1	2.73	0.102

Step 2 (Removed Compact Myocardium Thickness):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	4.16	0.054
Ploidy	1	3.29	0.081
Initial O_2	1	0.04	0.844
Condition Factor	1	0.84	0.360
Relative Ventricular Mass	1	1.23	0.267
Hepatosomatic Index	1	1.38	0.241
Interlamellar Cell Mass	1	0.24	0.625
Hematocrit	1	1.84	0.176
O_2 Acclimation × Ploidy	1	0.70	0.413
O_2 Acclimation × Initial O_2	1	0.98	0.324
Ploidy × Initial O_2	1	0.62	0.434
Acclimation × Ploidy × Initial O_2	1	2.82	0.097

Step 3 (Removed Interlamellar Cell Mass):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	4.26	0.051
Ploidy	1	3.80	0.062
Initial O_2	1	0.05	0.821
Condition Factor	1	0.79	0.376
Relative Ventricular Mass	1	1.20	0.276
Hepatosomatic Index	1	1.33	0.250
Hematocrit	1	1.80	0.181
O_2 Acclimation × Ploidy	1	0.72	0.405
O_2 Acclimation × Initial O_2	1	1.05	0.308
Ploidy × Initial O_2	1	0.66	0.420
Acclimation × Ploidy × Initial O_2	1	2.77	0.100

Step 4 (Removed Condition Factor):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	4.44	0.048
Ploidy	1	2.98	0.098
Initial O_2	1	0.06	0.814
Relative Ventricular Mass	1	1.18	0.278
Hepatosomatic Index	1	1.62	0.205
Hematocrit	1	1.70	0.194
O_2 Acclimation × Ploidy	1	0.87	0.362
O_2 Acclimation × Initial O_2	1	0.91	0.343
Ploidy × Initial O_2	1	0.48	0.488

Acclimation × Ploidy × Initial O_2	1	3.00	0.087

Step 5 (Removed Relative Ventricular Mass):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	5.70	0.027
Ploidy	1	2.79	0.109
Initial O_2	1	0.06	0.817
Hepatosomatic Index	1	2.50	0.115
Hematocrit	1	2.57	0.111
O_2 Acclimation × Ploidy	1	0.94	0.343
O_2 Acclimation × Initial O_2	1	1.38	0.243
Ploidy × Initial O_2	1	0.37	0.543
Acclimation × Ploidy × Initial O_2	1	2.93	0.091

Step 6 (Removed Hepatosomatic Index):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	6.16	0.022
Ploidy	1	4.44	0.048
Initial O_2	1	0.11	0.746
Hematocrit	1	4.55	0.034
O_2 Acclimation × Ploidy	1	1.01	0.328
O_2 Acclimation × Initial O_2	1	1.72	0.194
Ploidy × Initial O_2	1	0.17	0.680
Acclimation × Ploidy × Initial O_2	1	2.91	0.092

Step 7 (Removed O_2 Acclimation × Ploidy × Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	6.05	0.022
Ploidy	1	4.38	0.049
Initial O_2	1	0.11	0.747
Hematocrit	1	4.15	0.043
O_2 Acclimation × Ploidy	1	0.99	0.331
O_2 Acclimation × Initial O_2	1	1.72	0.193
Ploidy × Initial O_2	1	0.17	0.684

Step 7 (Removed Ploidy × Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	6.06	0.022
Ploidy	1	4.34	0.049
Initial O_2	1	0.11	0.744
Hematocrit	1	4.20	0.042
O_2 Acclimation × Ploidy	1	1.00	0.329
O_2 Acclimation × Initial O_2	1	1.79	0.184

Step 8 (Removed O_2 Acclimation × Ploidy):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	6.09	0.021
Ploidy	1	4.36	0.048
Initial O_2	1	0.12	0.735
Hematocrit	1	3.98	0.048
O_2 Acclimation × Initial O_2	1	1.74	0.191

Step 9 (Removed O_2 Acclimation × Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	6.39	0.019
Ploidy	1	4.76	0.040
Initial O_2	1	0.10	0.756
Hematocrit	1	4.04	0.046

Step 10 (Removed Initial O_2):

Source of variation	df	F-value	*p*-value
O_2 Acclimation	1	6.47	0.018
Ploidy	1	4.83	0.038
Hematocrit	1	4.04	0.046

Curriculum Vitae

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MSc. (Biology) University of New Brunswick, Fredericton, NB, 2020-2022
BEd. (Secondary Education) University of New Brunswick, Fredericton, NB, 2018-2019
BSc. Honours (Biology) Mount Allison University, Sackville, NB, 2014-2018

Conference Presentations:

McGeachy SA*, Benfey TJ. August 2022. Investigating hypoxia tolerance of triploid brook charr (*Salvelinus fontinalis*). Aquaculture Canada and WAS North America Conference (St. John's, Newfoundland and Labrador).

McGeachy SA*, Benfey TJ. May 2022. Investigating hypoxia tolerance of triploid brook charr (*Salvelinus fontinalis*). 28th Annual University of New Brunswick Graduate Research Conference (Virtual).

McGeachy SA*, Benfey TJ. May 2022. Investigating hypoxia tolerance of triploid brook charr (*Salvelinus fontinalis*). 61st Annual Canadian Society of Zoologists Conference (Virtual).

McGeachy SA*, Benfey TJ. May 2021. Improving hypoxia tolerance of triploid brook charr (*Salvelinus fontinalis*). 60th Annual Canadian Society of Zoologists Conference (Virtual).

McGeachy SA*, Benfey TJ. November 2020. The effect of chronic hypoxia and temperature on hypoxia tolerance in brook charr (*Salvelinus fontinalis*). Aquaculture Association of Canada Virtual Student Conference 2020 (Virtual).