Received: 17 November 2024 | Revised: 30 March 2025 | Accepted: 14 May 2025

DOI: 10.1111/jfb.70100

REGULAR ARTICLE

JOURNAL OF FISH BIOLOGY fsbi

The physiological response of juvenile diploid and triploid Arctic charr *Salvelinus alpinus* to exhaustive exercise

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Funding information
Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: RGPIN-2023-04628

Abstract

Triploidy is an effective tool for producing sterile fishes but often results in impaired performance in commercial aquaculture. In light of this, our study compared the physiological response to exhaustive exercise in juvenile diploid and triploid Arctic charr *Salvelinus alpinus*, a polar species with great potential for aquaculture. A standard ramping swimming protocol revealed no significant difference in critical swimming velocity (U_{crit}) between ploidies. There was also no effect of ploidy on post-U_{crit} blood glucose, lactate or haematocrit. However, triploids had a significantly higher frequency of erythrocyte nuclear segmentation. Independent of ploidy, there was also a significant positive correlation between blood lactate levels and U_{crit}. We conclude that triploidy does not impair the response to exhaustive exercise in juvenile *S. alpinus*.

KEYWORDS
aquaculture, erythrocyte, exercise, polar, salmonid, triploidy

1 | INTRODUCTION

The Arctic charr *Salvelinus alpinus* L. is a cold-water salmonid species native to Arctic, boreal and temperate regions of the northern hemisphere with great aquaculture potential due to its tolerance of high stocking densities, low optimum rearing temperature and high fillet quality and yield (Sæther et al., 2013; Yossa et al., 2019). However, farmed *S. alpinus* are prone to preharvest sexual maturation, a process which diverts energy allocation from somatic growth to the gonads, thereby decreasing fillet yield and quality, and increases disease susceptibility (Yossa et al., 2019). Additionally, it creates the risk of genetic introgression of the domesticated genome into locally adapted wild populations in the event of farm escapes, as documented for farmed Atlantic salmon *Salmo salar* (Glover et al., 2017; Wacker et al., 2023; Wringe et al., 2018).

A practical solution to unwanted sexual maturation of farmed fishes is to use functionally sterile triploid populations (Benfey, 2016; Piferrer et al., 2009). However, extensive pilot-scale and commercial trials with triploid *S. salar* have identified numerous welfare and farm production concerns when compared to diploids, including cataracts, skeletal deformities and overall reduced survival (Fraser et al., 2012; Madaro et al., 2022; O'Flynn et al., 1997). Although there have been fewer such studies of triploid *S. alpinus*, there is similar evidence of reduced survival (Chiasson et al., 2009; Fraser et al., 2022) and higher incidence of vertebral deformities (Fraser et al., 2022). Many studies have also shown triploid salmonids to have a reduced ability to tolerate environmental stressors such as high temperature and hypoxia (e.g. Benfey & Devlin, 2018; Hansen et al., 2015; Jensen & Benfey, 2022; Sambraus et al., 2017, 2018; Scott et al., 2015), possibly due to reduced aerobic scope at elevated temperatures (Riseth et al., 2020). There are, however, conflicting findings in the literature: at least two studies have failed to find such an effect of triploidy on aerobic scope or maximum metabolic rate at elevated temperatures (Bowden et al., 2018; Sezaki et al., 1991).

Increased erythrocyte size is often suggested as the limiting factor to the respiratory capacity of triploids because this results in

reduced surface area relative to volume compared to the smaller erythrocytes of diploids (Benfey, 1999; Benfey & Devlin, 2018). Triploid salmonids also frequently exhibit a higher incidence of erythrocyte nuclear segmentation (ENS) (Clark et al., 2025; Dorafshan et al., 2008; Wang et al., 2010; Wlasow et al., 2014, 2004), a cellular deformity of unknown origin that is defined as the medial division of the nucleus (Yokote, 1982). This deformity has yet to be reported in *S. alpinus*.

Routine aquaculture operations such as vaccination and grading require fish to be crowded for short periods of time, often in combination with capture (by netting) and removal from the water. Triploid salmonids generally respond well to this in terms of their primary and secondary stress response (Benfey & Biron, 2000; Biron & Benfey, 1994; Hyndman et al., 2003a; Madaro et al., 2024; Preston et al., 2017; Sadler et al., 2000) although triploids may exhibit high mortality if pushed to exhaustion at high temperature (Hyndman et al., 2003b).

Critical swimming velocity (U_{crit}) is a simple test frequently used to assess physiological responses to exhaustive exercise in fishes. It is positively correlated with haematocrit and blood haemoglobin levels (Pearson & Stevens, 1991), cardiac output (Clark & Seymour, 2006) and metabolic rate (Horodysky et al., 2011; Norin & Clark, 2016), and overall reflects maximum aerobic capacity (Kolok, 1999; Norin & Clark, 2016; Plaut, 2001). While previous studies have shown that U_{crit} is not affected by triploidy in several salmonid species (Bernier et al., 2004; Lijalad & Powell, 2009; Riseth et al., 2020; Scott et al., 2015; Small & Randall, 1989; Stillwell & Benfey, 1997), this has not been examined in *S. alpinus*.

In this study, we examine the effects of triploidy on the response of this polar species to exhaustive exercise through a U_{crit} test with post-exercise blood sampling. Based on previous studies in other salmonids, we hypothesized that U_{crit} would not differ between diploids and triploids. We sought to supplement this information with measurements of secondary stress response (blood glucose, lactate and haematocrit), and to investigate the presence, and potential causes, of ENS in *S. alpinus*. Such information can assist in developing species-specific farm management practices for this relatively understudied salmonid aquaculture species.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This research was approved by the University of New Brunswick (UNB) Animal Care Committee (Animal Use Protocol 24007) and followed all applicable welfare and experimental guidelines of the Canadian Council on Animal Care.

2.2 | Fish

Diploid and triploid *S. alpinus* embryos were purchased at the eyed-egg stage from the Valorēs breeding program (Shippagan, NB, Canada). They were progeny of three dams (pooled eggs) and 10 sires (pooled milt) artificially spawned in April 2023, with triploidy induced in approximately half of the eggs via hydrostatic pressure treatment of 5 min at 65.5 MPa beginning 210°C-min post-fertilization and the remainder retained as diploid controls. Survival to the eyed stage (prior to shipping) was 58 ± 1% for triploids and 77 ± 1% for diploids. Fish were subsequently reared at the UNB aquatic facility (Fredericton, NB, Canada) following standard husbandry procedures (Jobling et al., 2010), with diploids and triploids reared separately in flow-through tanks supplied with dechlorinated municipal water. Mortality at UNB was not recorded but was low and with no obvious difference between ploidies.

In January 2024, all fish were moved to 314-L ploidy-specific tanks within a six-tank recirculating aquaculture system (RAS) for the months preceding experimentation. Water quality parameters in this RAS were maintained within recommended ranges (total ammonia nitrogen and nitrite both <1.0 mg/L, nitrate 0–400 mg/L, alkalinity 50–300 mg/L, hardness >100 mg/L and pH 6.5–8.5; Timmons et al., 2018) with the exception of low alkalinity (43 ppm) on a single day that was immediately corrected by increasing makeup water to the RAS. Temperature and dissolved oxygen averaged 10.3°C ± 1.0°C and 93% ± 5% of air saturation, respectively, for the entire time in the RAS, including through to the end of the experimental period. Stocking density was approximately 109 kg m^{-3} at the time of experimentation, with individuals weighing approximately 160 g on average (Table 1). All fish used for this experiment came from a single diploid and single triploid tank. Twenty days prior to experimentation, the seasonally adjusted photoperiod in the room was increased to 13:11 h (light:dark) from 12:12 to mimic the seasonal trends of early summer for the sake of broodstock maintained within a separate RAS, and this 13:11 regime was maintained throughout the experimental period.

2.3 | Critical swimming velocity protocol

Trials began on 5 June 2024 and continued for 24 consecutive days. One trial was completed per day, alternating between a triploid and diploid fish, for a total of 12 fish per ploidy. A submerged 30-L swim tunnel (Loligo Systems) was used to determine U_{crit}, with velocity calibrated via a flow meter (Streamflo 430; Nixon Flowmeters). A submersible pump was used in tandem with an open port, both located immediately before the motor driven propeller, to continuously exchange fresh water from the outer tank into the swim tunnel (10.7 L min^{-1}). A constant flow of aerated water from an adjacent head tank was pumped through the outer tank to mitigate any heat generated by the swimming apparatus and to ensure stable oxygen levels. Dissolved oxygen (Pro20; YSI) and temperature (Traceable 4015; Cole-Parmer) were measured in the outer tank. Average temperature and dissolved oxygen levels in the swim tunnel setup over the 24-day test period were 14.9°C ± 0.7°C and 98% ± 2% of air saturation, respectively.

A foam board with a reflective surface was clamped to the exterior face of the outer tank to prevent visual stimuli from influencing swimming behaviour. A self-adhesive ruler within the swimming chamber was used to estimate each fish's fork length (L_F) and thereby calculate

TABLE 1 Critical swimming velocity (U_{crit}) and parameters measured immediately after fish reached U_{crit} in juvenile diploid (2*n*) and triploid (3*n*) *Salvelinus alpinus* (mean ± standard deviation, $n = 12$ per ploidy).

Parameter	2*n*	3*n*	*df*	*t*	*p*
U_{crit} ($L_F s^{-1}$)	11.47 ± 2.30	10.95 ± 1.45	18.574	0.6639	0.5149
Body mass (g)	158.99 ± 14.59	162.06 ± 20.44	19.865	−0.42322	0.6767
Fork length (L_F; cm)	23.60 ± 1.16	23.92 ± 1.07	21.879	−0.71383	0.4829
Condition factor	1.21 ± 0.08	1.19 ± 0.15	17.354	0.52929	0.6033
Hepatosomatic index (%)	1.31 ± 0.13	1.30 ± 0.19	19.412	0.10873	0.9145
Gonadosomatic index (%)	0.06 ± 0.06	0.03 ± 0.01	12.409	1.9989	0.0680
Haematocrit (%)	39.99 ± 2.78	39.64 ± 3.75	20.279	0.256	0.8005
Glucose (mmol L^{-1})	3.08 ± 0.48	3.20 ± 0.67	20.002	−0.49021	0.6293
Lactate (mmol L^{-1})	3.38 ± 1.22	4.24 ± 2.01	18.137	−1.2644	0.2221
	2*n*	**3*n***	***df***	**χ^2**	***p***
ENS (%)	0.83 ± 1.34	9.83 ± 4.30	1	17.551	**<0.0001**

Note: Welch's *t*-test was used for all parameters except erythrocyte nuclear segmentation (ENS) frequency, where the Kruskal–Wallis test was used. *df*, degrees of freedom; t = Welch's *t*-statistic; χ^2, Kruskal–Wallis chi-squared value; *p*, probability value, with significant *p* values in bold).

swimming velocity ($L_E s^{-1}$) to minimize handling stress before beginning the experiment. This L_E overestimated the true fork length (L_F; measured after the swimming trial was completed) by an average of 3.9%. Fish were observed from above through the transparent lid of the swimming chamber, with special care taken not to startle the fish.

Swimming trials began between 0900 and 0920 every day with the fish of the day haphazardly chosen from its stock tank and placed in the swimming chamber (dimensions 55 × 14 × 14 cm) of the swim tunnel. The remaining fish in that stock tank were fed immediately after removing the test fish, thereby ensuring that feed was always withheld from each stock tank for 24 h prior to selecting a fish for experimentation. Once placed in the swim tunnel, the fish was given a 1-h habituation period at a velocity of 1.0 $L_E s^{-1}$ and then the velocity was increased by 1.0 $L_E s^{-1}$ every 10 min, following the ramping protocol of Scott et al. (2015). This continued until the fish was exhausted, which was defined as inability to swim against the current and being forced onto the metal grate at the end of the swimming chamber for at least 3 s. Unlike Scott et al. (2015), we did not swim the fish a second time as part of the U_{crit} protocol; rather, the time and swimming velocity were recorded, the motor was switched off, and the fish was immediately removed from the swim tunnel and anaesthetized with benzocaine (ethyl-4-aminobenzoate, 0.05 g L^{-1}) in an aerated 3-L bath. Fish remained motionless on the chamber floor after the motor was turned off, displaying signs of exhaustion such as rapid opercular movements and reacting minimally to handling. Two of the 24 fish (both triploids) lost equilibrium at U_{crit}; these fish were allowed to recover in the swim tunnel with no flow before removal and subsequent anaesthesia.

2.4 | Post-exercise sampling

On anaesthesia, fish were weighed and had their fork length (L_F; note that this value is used in subsequent analysis instead of the initial L_E estimate), body width and height measured, with these last two measurements made just anterior to the dorsal fin. Blood was then drawn from the caudal vasculature via heparinized needle and syringe (23G and 1 mL, respectively), after which fish were euthanized via benzocaine overdose (0.5 g L^{-1}) followed by severing of the spinal connection at the skull. The blood was then immediately processed to measure glucose (FreeStyle Lite Blood Glucose Monitoring System; Abbott) (Ball & Weber, 2017) and lactate (Lactate Plus Meter; Nova Biomedical) (Vaage et al., 2023), to prepare a blood smear and to determine haematocrit. The liver and gonads were then removed and weighed to determine the hepatosomatic (I_H) and gonadosomatic (I_G) indices, and the gonads were visually assessed to determine sex. Body mass and L_F data were used to calculate Fulton's condition factor (*K*) as per Ricker (1975):

$$K = \text{mass} \times (L_F)^{-3} \times 100$$

Body width and height data were used to calculate each fish's cross-sectional area to check whether correction for a solid blocking effect in the swim chamber would be necessary, that is, if greater than 10% of the cross-sectional area of the swimming chamber (196 cm^2) (Bell & Terhune, 1970). Fish cross-sectional area was 6.43% ± 0.73% (maximum 7.96%) of the swimming chamber cross-sectional area and we therefore did not correct for a blocking effect. Blood smears were later analysed to confirm ploidy level (Yossa et al., 2018; average length of 30 erythrocytes per fish) and to determine ENS frequency (Yokote, 1982; 50 erythrocytes per fish).

2.5 | Critical swimming velocity calculation

Critical swimming velocity (U_{crit}; $L_F s^{-1}$) was calculated as per Brett (1964):

$$U_{crit} = U_f + \left(U_i \times \left[t_f \times t_i^{-1}\right]\right)$$

where U_f is the final velocity achieved ($L_F s^{-1}$), t_f is the time spent swimming at that velocity (*s*), U_i is the change in swimming velocity at

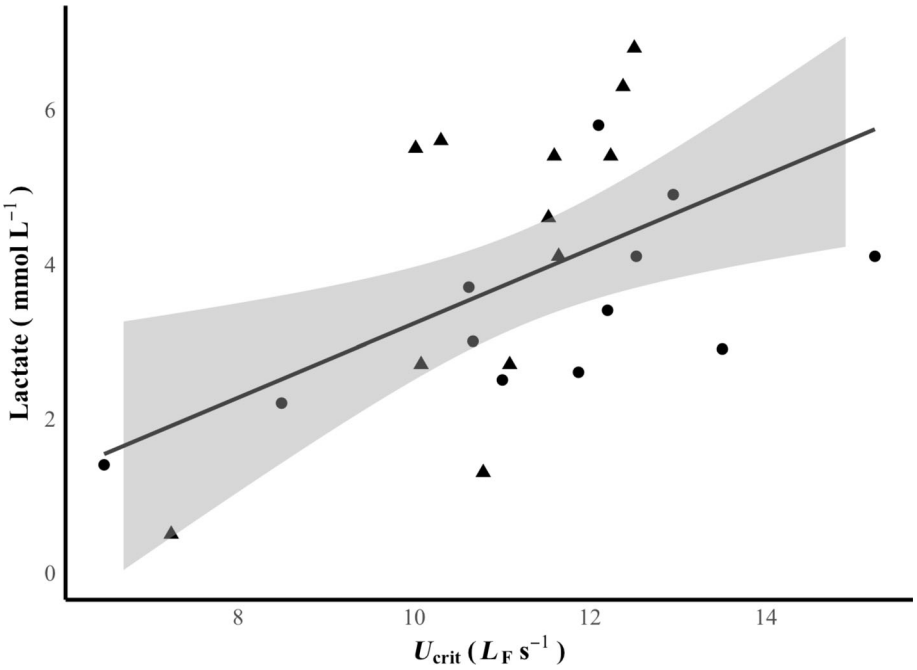

FIGURE 1 Significant positive correlation between critical swimming velocity (U_{crit}, L_F = fork length) and post-exercise blood lactate levels in juvenile diploid (circles; $n = 12$) and triploid (triangles; $n = 12$) *Salvelinus alpinus*. Solid line is linear regression ($r = 0.541$, $p < 0.01$, $y = 0.4803x - 1.5706$) with 95% confidence interval shaded. Blood lactate levels were not different between ploidies, therefore this correlation includes the diploid and triploid fish.

each increment ($L_F s^{-1}$) and t_i is the duration of each increment (s). Note that this calculation used true L_F (measured on completion of the swimming test) rather than the initial L_E estimate.

2.6 | Statistical analyses

Data are reported as mean ± standard deviation. Inferential statistics were obtained using R version 4.4.2 (R Core Team, 2022) with 'readxl' (Wickham & Bryan, 2023) and 'ggplot2' (Wickham, 2016) packages and using a significance level of $\alpha = 0.05$. Separate Welch's t-tests were used to determine the statistical significance of ploidy differences in body mass, L_F, K, haematocrit, glucose, lactate, I_H, I_G and U_{crit}. The Kruskal–Wallis test was used to assess the ploidy effect on ENS frequency due to non-normal distribution of the data.

Separate Pearson correlation analyses were performed to assess linear relationships between U_{crit} and each of body mass, L_F, K, haematocrit, glucose, lactate, I_H, I_G and ENS frequency. Ploidy-specific correlation tests were performed when initial Welch's t-tests or the Kruskal–Wallis test revealed a significant ploidy effect.

3 | RESULTS

All 24 fish were confirmed to be their presumed ploidy. The diploid group was composed of five males and seven females, and the triploid group of seven males and five females, and all fish were clearly juvenile (I_G <0.1%). Fish were able to turn around and explore their surroundings during the initial 1-h habituation period but then exhibited burst-and-glide locomotion once moderate swimming velocities were achieved. Fish generally preferred swimming at the right upstream corner of the swimming chamber until approaching U_{crit}, when position became highly variable. Of the two triploids that lost equilibrium at U_{crit}, one lost equilibrium at the second lowest velocity and the other at the third highest out of the 12 triploids.

The only parameter that was affected by ploidy was ENS frequency (significantly higher in triploids; Table 1). ENS did not correlate with U_{crit} in either diploids ($p > 0.1$, $r = -0.077$) or triploids ($p > 0.1$, $r = -0.337$). Blood lactate was the only parameter that had a significant correlation with U_{crit} (Figure 1).

4 | DISCUSSION

4.1 | Critical swimming velocity

Our study investigated the effects of triploidy on the response of juvenile *S. alpinus* to exhaustive exercise through a ramping U_{crit} test. In line with previous studies that have measured U_{crit} in triploids of other salmonid species (Bernier et al., 2004; Lijalad & Powell, 2009; Riseth et al., 2020; Scott et al., 2015; Small & Randall, 1989; Stillwell & Benfey, 1997), we found no significant ploidy effect on U_{crit}. However, it is notable that in almost all cases, triploids have a slightly lower U_{crit} than diploids (Table 2). This suggests that there may be a subtle impact of triploidy on performance in exhaustive exercise challenges that is not apparent within individual studies. This may be an outcome of the reduced surface area of triploid erythrocytes (relative to their volume) constraining aerobic capacity of triploid fish under conditions of high oxygen demand, as hypothesized by Benfey and Devlin (2018). Given that triploid salmonids likely have a lower thermal optimum than conspecific diploids (Atkins & Benfey, 2008; Hansen et al., 2015; Riseth et al., 2020; Sambraus

TABLE 2 Published values for mean critical swimming velocity ([fork length] s^{-1}) in diploid (2*n*) and triploid (3*n*) salmonids, with the additional calculation of 3*n* values as a proportion of 2*n* (3*n*/2*n*).

Species	2n	3n	3n/2n	Reference
Oncorhynchus kisutch	3.66	3.45	0.943	Small & Randall, 1989
Salvelinus fontinalis (1994 cohort)	2.31	2.16	0.935	Stillwell & Benfey, 1997
Salvelinus fontinalis (1995 cohort)	1.86	1.73	0.930	Stillwell & Benfey, 1997
O. tshawytscha	3.92	3.60	0.918	Bernier et al., 2004
Salmo salar (1st test)	2.97	3.00	1.010	Lijalad & Powell, 2009
Salmo salar (2nd test)	2.97	2.96	0.997	Lijalad & Powell, 2009
O. mykiss (BW strain, 2008 BY)	5.03	5.48	1.089	Scott et al., 2015
O. mykiss (TZ strain, 2008 BY)	5.53	5.02	0.907	Scott et al., 2015
O. mykiss (PN strain, 2008 BY)	6.20	6.05	0.976	Scott et al., 2015
O. mykiss (BW strain, 2009 BY)	8.27	8.18	0.990	Scott et al., 2015
O. mykiss (TZ strain, 2009 BY)	8.58	8.40	0.980	Scott et al., 2015
O. mykiss (PN strain, 2009 BY)	9.58	8.95	0.935	Scott et al., 2015
O. mykiss (FV strain, 2009 BY)	10.77	10.88	1.011	Scott et al., 2015
Salmo salar (3°C)	1.51	1.49	0.987	Riseth et al., 2020
Salmo salar (10.5°C)	2.14	1.88	0.879	Riseth et al., 2020
Salvelinus alpinus	11.47	10.95	0.955	This study

Note: All values for Scott et al. (2015) are estimates made from family means shown in their Figure 1.
Abbreviation: BY, brood year.

et al., 2017, 2018; Verhille et al., 2013), it would be useful to assess U_{crit} across a range of temperatures within the same populations of diploids and triploids. To our knowledge, Riseth et al. (2020) are the only ones to have done this to date. They found no statistically significant effect of temperature on U_{crit} among or between diploid and triploid *S. salar*, despite triploids generally showing a reduced capacity for swimming at high temperature as indicated by an earlier onset of ram ventilation when swimming at 10.5°C.

Because all fish had the same preferred swimming location in the swim tunnel, we presume that there was some minor disruption in laminar flow and thus potentially an overestimation of U_{crit} in our experiment. A similar observation was reported in the U_{crit} trials of Hvas and Oppedal (2019) with their 90 L Loligo swim tunnel, although at a different region of the swimming chamber. As such, we suggest that the critical swimming velocities reported in the present study be used to compare the relative performance of diploids and triploids only and not taken as absolute values for the species. This is especially evident when comparing the performance of our juvenile diploid *S. alpinus* to those studied in Pettersson et al. (2010), where their estimates of U_{crit} at similar temperatures (10 and 17°C) were approximately 4 $L_F s^{-1}$. However, this difference in U_{crit} may not be entirely due to a disruption of laminar flow. For instance, differences in U_{crit} ramping protocol (i.e. the time interval between increasing speeds) can affect the maximal swimming velocity achieved. This is generally thought to be an inverse relationship, where an increased time interval results in a decreased U_{crit} (Farlinger & Beamish, 1977; Kolok, 1999). In our study, we followed the ramping protocol of Scott et al. (2015) by increasing the swimming velocity by 1.0 fork length every 10 min. In their 10 and 17°C treatments Pettersson et al. (2010) used the different approach of Jain et al. (1997), where fish were brought to 75% of their estimated U_{crit} and then swimming velocity was increased slowly by 0.4 body lengths every 30 min. Our faster U_{crit} ramping protocol likely contributes to our higher U_{crit} values, despite having fish of similar size to Pettersson et al. (2010). Illing et al. (2021) found species-specific differences in the relationship between ramping protocol and U_{crit} among small marine fishes; a future study explicitly testing different ramping protocol procedures in *S. alpinus* or another salmonid could further clarify this effect.

4.2 | Secondary stress response

Post-exercise analysis revealed no ploidy effect on haematocrit, blood glucose and blood lactate levels immediately after exhaustion. The absence of a ploidy effect on these secondary responses to acute stress in salmonids during the post-exercise recovery period has previously been reported in some studies (Benfey & Biron, 2000; Bernier et al., 2004; Biron & Benfey, 1994; Hyndman et al., 2003a; Sadler et al., 2000), but others have observed higher blood glucose and lactate levels in triploid salmonids compared to diploids at multiple time points prior to full recovery (Lahnsteiner et al., 2019; Madaro et al., 2024; Preston et al., 2017). Despite the absence of a ploidy effect on blood lactate, we found a significant positive correlation between U_{crit} and blood lactate levels, indicating that fish with a higher U_{crit} also generally had a higher level of post-exercise blood lactate. This positive relationship, which has only previously been demonstrated in diploids (rainbow trout *Oncorhynchus mykiss*; Jain & Farrell, 2003), reflects the high reliance on anaerobic metabolism at U_{crit} in salmonids (Birnie-Gauvin et al., 2023).

4.3 | Erythrocyte nuclear segmentation

In line with other studies on salmonids (Clark et al., 2025; Dorafshan et al., 2008; Wang et al., 2010; Wlasow et al., 2014; Własow et al., 2004), we found a significantly higher frequency of ENS in triploids. To our knowledge, this is the first report of ENS in *S. alpinus*. The cause of ENS remains elusive, but one theory is that it is due to repeated mechanical stress as erythrocytes are forced through narrow capillaries that are no wider than in diploids despite having larger erythrocytes (Small et al., 2024). We found no significant correlation between ENS and U_{crit}, leading us to conclude both that ENS frequency is not affected by exhaustive exercise and, conversely, that swimming ability is not affected by ENS frequency in this species.

While the cause of ENS is unclear, so too are its effects on erythrocyte function. Erythrocytes are known to swell under instances of oxygen demand (Houston, 1997; Witeska, 2013), and with U_{crit} known to illicit maximum aerobic metabolic rate and oxygen demand (Norin & Clark, 2016; Plaut, 2001), one would expect any inhibition of erythrocyte function to become apparent during a U_{crit} test. However, the present study provides no evidence that ENS limits erythrocyte function as triploids did not have a lower U_{crit}, despite a nearly 10-fold higher ENS frequency when compared to diploids. A more direct analysis of erythrocyte function is likely necessary to truly measure the impact of ENS on the cellular level.

5 | CONCLUSION

In conclusion, triploidy has no apparent effect on the critical swimming velocity or indices of the secondary stress response at exhaustion of *S. alpinus*. While triploids of this species have the same characteristic increase in ENS observed in other species, it does not correlate with U_{crit}. Our findings indicate that triploidy induction in *S. alpinus* to produce sterile populations should not affect their ability to withstand aquaculture procedures that involve acute, strenuous exercise.

AUTHOR CONTRIBUTIONS

J.D.C.: Conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing – original draft. **T.J.B.:** Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review & editing.

ACKNOWLEDGEMENTS

This research was completed in partial fulfilment of the requirements of an undergraduate senior research project in Biology at UNB. We are grateful to Abigail Concannon (UNB Biology) for her assistance in the execution of this experiment and in fish care. We also thank Katy Haralampides and Dennis Connor (UNB Civil Engineering) for loan of the flow meter and instruction on its use, Heather Burke, Katie Theriault, Megan Fraser and Chandler Stairs (UNB Aquatic Facility) for excellent fish care and for their assistance in troubleshooting, Jacob Wickens for his assistance during the experiment, and the Natural Sciences and Engineering Research Council of Canada for funding. We respectfully acknowledge that the Fredericton campus of UNB (where this research took place) stands on unsurrendered and unceded traditional Wolastoqey land.

FUNDING INFORMATION

Funding awarded by the Natural Sciences and Engineering Research Council of Canada to TJB (Discovery Grant RGPIN-2023-04628).

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How to cite this article: Clark, J. D., & Benfey, T. J. (2025). The physiological response of juvenile diploid and triploid Arctic charr *Salvelinus alpinus* to exhaustive exercise. *Journal of Fish Biology*, 1–8. https://doi.org/10.1111/jfb.70100