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ARTICLE

Diploid and triploid white sturgeon (*Acipenser transmontanus*) differ in magnitude but not kinetics of physiological responses to exhaustive exercise at ambient and elevated temperatures

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Abstract: Triploid salmonids have been shown to underperform in suboptimal environments. It is thought this might be due to having larger cells to accommodate the increased number of chromosomes and therefore effects on aerobic metabolism from having smaller cellular surface area to volume ratios. The goal of this study was to examine the aerobic metabolism of diploid and triploid white sturgeon (*Acipenser transmontanus*) in ambient (18 °C) and elevated water temperatures (24 °C). Resting and maximum metabolic rates, recovery time from exhaustive exercise, and surface area to volume ratios of erythrocytes and their nuclei in diploid and triploid sturgeon were evaluated. Triploid sturgeon had a reduced aerobic scope and hematological response (hematocrit and hemoglobin) to exhaustive exercise. A reduced surface area to volume ratio of erythrocytes in triploid sturgeon provides evidence that cellular surface area could be one mechanism limiting aerobic metabolism in triploid fishes. A lower aerobic scope found in triploid sturgeon may impact reproductive and somatic growth, yet more research is needed to determine implications for management decisions on farms and hatcheries.

Résumé : Il a été démontré que la performance de salmonidés triploïdes dans des milieux suboptimaux est moins bonne que celle de salmonidés diploïdes. Cela pourrait être dû au fait qu'ils ont des cellules plus grosses pour contenir leur plus grand nombre de chromosomes, ce qui aurait des effets sur le métabolisme aérobie associés aux rapports plus faibles de la surface et du volume des cellules. Le but de l'étude était d'examiner le métabolisme aérobie d'esturgeons blancs (*Acipenser transmontanus*) diploïdes et triploïdes dans des températures ambiante (18 °C) et élevée (24 °C) de l'eau. Les métabolismes de base et maximum, le temps de récupération d'un exercice épuisant et les rapports de la surface et du volume d'érythrocytes et de leurs noyaux chez des esturgeons diploïdes et triploïdes ont été évalués. L'exercice intensif s'est traduit par une amplitude aérobie et une réaction hématologique (hématocrite et hémoglobine) réduites chez les esturgeons triploïdes. Un rapport plus faible de la surface et du volume des érythrocytes chez les esturgeons triploïdes indique que la surface des cellules pourrait être un facteur qui limite le métabolisme aérobie chez les poissons triploïdes. L'amplitude aérobie plus faible observée chez les esturgeons triploïdes pourrait avoir une incidence sur la croissance reproductive et somatique, mais plus de travaux sont nécessaires afin d'en établir l'importance en ce qui concerne les décisions de gestion pour les pisciculteurs et les écloseries. [Traduit par la Rédaction]

Introduction

Study of the relationship between ploidy and physiological performance in fishes, at any biological level, has thus far been mostly limited to salmonid species (see Benfey 1999 and Maxime 2008 for reviews). The production of triploid salmonids in aquaculture provides the advantage of sterility. While there are mixed results about growth differences in diploid and triploid salmonids, in general, diploid and triploid fishes exhibit similar growth before sexual maturation, and triploid salmonids demonstrate superior growth at the time diploid fish undergo sexual maturation (reviewed by Benfey 1999). Beyond differences in growth, triploidy in salmonids has been shown to affect other aspects of cellular and organismal physiology. For example, triploid salmonids have larger but fewer cells (Small and Benfey 1987). Additionally, it has been commonly reported that triploid salmonids exhibit poorer performance in low oxygen or high oxygen demanding environments compared with their diploid counterparts (reviewed in Maxime 2008). The poorer performance demonstrated by triploid salmonids in suboptimal environments, such as high water temperatures (Ojolick et al. 1995; Altimiras et al. 2002; Hyndman et al. 2003*a*; Hansen et al. 2015; Sambraus et al. 2017), may be due to a difference in aerobic metabolic capacity between diploid and triploid salmonids. Altimiras et al. (2002) found a reduced factorial aerobic scope in triploid brown trout (*Salmo trutta*), while O'Donnell et al. (2017) demonstrated a higher standard metabolic rate in triploid brook char (*Salvelinus fontinalis*), implying a reduced aerobic scope. Moreover, multiple studies have suggested a reduction in surface area to volume ratio (SA:*V*) in triploid cells and nuclei as an explanation for differences in aerobic performance between diploid and triploid fishes (Benfey 1999; Sadler et al. 2001; Ballarin et al. 2004; Leal et al. 2019). Cellular SA:*V* directly affects biochemical processes, such as diffusion (Okie 2013); therefore, a reduction in cellular and nuclear SA:*V* may limit diffusion of oxygen, which can hinder oxygen-limited processes like cellular respiration. In environments where metabolic demand is elevated, such as acute high water temperatures (Brett and Glass 1973; Moffitt and Crawshaw 1983), the reduction in SA:*V* in triploid fishes may impact aerobic capacity, thereby reducing their physiological performance. While many studies have proposed differences in SA:*V* as an explanation for differences in physiological performance in diploids and triploids, to our knowledge, none have quantified cellular and nuclear SA:*V* in diploid and triploid fishes.

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Despite the physiological differences in triploid salmonids, to date, there has been little research comparing the physiological effect of differences in ploidy in other fish species. In sturgeon, subtle differences in the stress response of diploid and triploid shortnose sturgeon (*Acipenser brevirostrum*; Beyea et al. 2005) and differences in cellular metabolic enzyme activity in diploid and triploid white sturgeon (*Acipenser transmontanus*; Leal et al. 2019) have been found. White sturgeon are ancestral octoploids and, in the diploid state, have eight genome copies (8N); triploid white sturgeon have a genome that is 50% larger than diploids and thus have twelve genome copies (12N) (Drauch Schreier et al. 2011). Salmonids are ancestral tetraploids and contain four copies (4N) of their genome in the diploid state (Johnson et al. 1987), and triploid salmonids are sterile (Benfey 1999). Sturgeon provide a unique opportunity to study the effects of ploidy on physiology because sturgeon triploids are fertile (Drauch Schreier et al. 2011), in contrast with infertile triploid salmonids, although triploid female sturgeon appear to require longer to mature (>9 years; J. Van Eenennaam and A. Schreier, unpublished data). Additionally, salmonid and sturgeon fishes inherently differ in their life history, ecology, and physiology. For example, sturgeon have a markedly lower swimming performance (Peake et al. 1997; McKenzie et al. 2001; Downie and Kieffer 2017) and differ in their metabolic demand (Kieffer et al. 2001).

Owing to the lack of studies assessing sturgeon triploid physiology, we sought to investigate the link between elevated temperature, cellular SA:*V*, and aerobic metabolism in diploid and triploid white sturgeon. The goal of this study was to assess the effect of ploidy and elevated temperature on resting and maximum metabolic rates and aerobic scope of white sturgeon, as well as to determine the time to recover from exhaustive exercise. We also sought to characterize the difference in SA:*V* of erythrocytes and nuclei in diploid and triploid white sturgeon. In environments where oxygen demand is greater (e.g., elevated temperatures and forced swimming), SA:*V* may limit diffusion and, therefore, diffusion-limited processes, such as aerobic metabolism. We predicted lower resting and maximum metabolic rates in triploid white sturgeon (compared with diploid white sturgeon), especially when acutely exposed to elevated temperatures. Additionally, recovery from exercise is an oxygen-dependent process (Kieffer 2000); hence, we also predicted a longer time to recovery in triploid white sturgeon compared with diploid white sturgeon following exhaustive exercise.

Materials and methods

Fish source and husbandry

Ovulated eggs and milt were collected from one 8N white sturgeon female and male domesticated brood stock from a Northern California farm. Spawning induction, fertilization, and de-adhesion of eggs followed standard procedures (Van Eenennaam et al. 2004), with some modifications. Once collected, ovulated eggs and milt were kept separate in oxygen-filled bags in an ice chest (15.5 ± 0.5 °C) and transported to the Putah Creek Hatchery Facility at the University of California, Davis, California, USA. Eggs were aged for 7 h in coelomic fluid prior to fertilization and also mixed vigorously during de-adhesion to increase the incidence of triploidy (Van Eenennaam et al. 2020). Fertilized eggs were incubated in McDonald jars in a flow-through hatchery system (15.5 ± 0.5 °C). After hatch, larvae were transported to and reared at the Center for Aquatic Biology and Aquaculture facilities at the University of California, Davis. Following yolk-sac depletion, fish experienced a typical feeding regime as described in Leal et al. (2018). At 2 months posthatch, ploidy of individual fish was verified using a Coulter counter (Fiske et al. 2019), and 8N and 12N fish were reared in separate tanks with flow-through well water (ambient temperature 18.5 ± 0.5 °C) until experimentation as juvenile fish (3 months posthatch; mean weight 13 g). All husbandry, handling, experimental protocols, and sampling procedures followed the protocol approved by the UC Davis Institutional Animal Care and Use Committee (Protocol No. 20117).

Respirometry

Intermittent-flow respirometry was used to determine routine metabolic rate, maximum metabolic rate, and the time to recover from exhaustive exercise (Chabot et al. 2016). Oxygen consumption rates were measured at either ambient (18 °C) or elevated (24 °C) temperatures. A total of four trials with eight fish per trial (four 8N and four 12N sturgeon) were conducted for each experimental temperature (n = 16 fish per temperature and ploidy). For each trial, fish were fasted 24 h then subsequently placed in individual circular floating plastic containers (13.5 cm diameter, 15 cm depth), fitted with fine mesh sides and a mesh lid inside a fiberglass tank (96 cm diameter, 142 L). Water temperature was either maintained at 18 °C for 2 h or increased from 18 to 24 °C over 2 h. After the 2 h temperature exposure at 18 °C or the 2 h ramp from 18 to 24 °C, fish were then moved to individual glass square jars with rounded edges, which served as respirometer chambers (mean volume 0.497 L), and submerged in an aerated insulated water bath (142 L) with submersible heaters to maintain a constant temperature. Each jar was placed in a square plastic container covered in black tape to prevent visual disturbance of neighboring fish. A UV filter was used in the water bath to minimize microbial growth and microbial respiration. Oxygen consumption was measured overnight for at least 18 h with a 10 or 8 min measurement period for ambient and elevated temperature groups, respectively, followed by a 13 min flush period. Oxygen levels during portions of closed respirometry never dropped below 80% air saturation (7.09 mg·L^{-1} at 18 °C and 6.29 mg·L^{-1} at 24 °C). The following morning, fish were individually removed from their respirometer and immediately chased by hand for 3 min, followed by a 15 s air exposure. Manually chasing has previously been used to determine postexercise maximum metabolic rates (Kieffer et al. 2001; Zhang and Kieffer 2017). Exhaustion was characterized by the fish no longer responding to manual chasing and loss of equilibrium. More specifically, when the sturgeon stopped swimming away when touched, they were flipped upside down such that their dorsal side was facing the bottom of the bucket. If they did not flip back over within 5 s, they were considered exhausted. In a pilot study, eight fish (four fish per ploidy) were chased until exhaustion and the time to exhaustion was recorded. The time to exhaustion was not significantly affected by ploidy (p = 0.40), and 3 min was sufficient to exhaust all eight fish. Fish were chased in an insulated bucket (29 cm diameter, 15 L) maintained at the experimental temperature and aerated with air stones. After chasing, fish were netted, exposed to air for 15 s, and placed back in their respective respirometers. Oxygen consumption was measured every 15 min (5 min measure, 10 min flush) for an additional hour after chasing to determine time to recover from chasing. Mass specific metabolic rate (MO_2) was calculated as follows:

$$\text{Metabolic rate}\left(\text{mg } O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\right) = (\Delta O_2 \times V)/(M \times T)$$

where ΔO_2 is the change in oxygen concentration over the measurement period (in mg O_2·L^{-1}), V is the volume of water in the respirometer (L), M is the mass of the fish (kg), and T is the length of time of the measurement period (h). Maximum metabolic rate (MMR) was taken as the MO_2 immediately following chasing, which corresponded to the highest recorded metabolic rate during recovery for each fish. Routine metabolic rate (RMR) was calculated from the tenth quantile of the measurements taken during the 18 h period before chasing. The use of comparative quantiles (i.e., p values that contain a predetermined percentage of the left tail of the distribution of all the metabolic rates over the

recorded period) has been used for estimation of RMR in fish (reviewed by Chabot et al. 2016; see Via et al. 1994 and Davis et al. 2018 for examples). Absolute aerobic scope was calculated as the difference in MMR and RMR, while factorial aerobic scope was calculated as MMR divided by RMR. The temperature coefficient (Q_{10}) was calculated as the ratio between the metabolic rates at the two temperatures raised to the tenth power divided by the difference in experimental temperatures.

Chase–recovery

To assess the time it took hematological parameters to recover from exhaustive exercise, we subjected 8N and 12N white sturgeon to 3 min manual chase protocol followed by 15 s air exposure as described previously. Time to recovery was examined at two temperatures: ambient (18 °C) and elevated (24 °C). A total of 320 fish were used in the chase–recovery experiment (n = 16 fish per time point, temperature, and ploidy). All fish were fasted 24 h prior to experimentation. Eight fish at a time were held in individual floating plastic containers (13.5 cm diameter, 15 cm depth) fitted with fine mesh sides and a mesh lid for 2 h before being chased. Containers were kept in a fiberglass tank (96 cm diameter, 142 L) and either maintained at 18 °C (ambient) for 2 h or acutely increased from 18 to 24 °C over 2 h (elevated) using submersible heaters. Fish were then individually chased by hand for 3 min in an insulated plastic bucket (29 cm diameter, 15 L) maintained at their respective temperatures and aerated with an air stone. After chasing, fish were netted, exposed to air for 15 s, and placed back in their respective containers and allowed to recover for 0 (no recovery, Time 0), 30, 60, or 150 min. After recovery, fish were euthanized with a lethal dose of sodium bicarbonate buffered tricaine methanesulfonate (MS-222, 500 mg·L^{-1}). Baseline measurements were taken after the 2 h temperature exposure, before the chasing protocol. Blood sampling, hematocrit, hemoglobin, mean erythrocyte hemoglobin concentration, glucose, and lactate protocols followed standard methods as described in Leal et al. (2018).

Erythrocyte and nuclear surface area to volume ratios

Thirty fish from each ploidy were euthanized with a lethal dose of MS-222. Blood samples were taken as described in Leal et al. (2018). Blood smears were prepared immediately after blood was drawn. Smears were air-dried for 24 h, fixed in methanol, and stained with Giemsa stain (Blaxhall and Daisley 1973). For each slide, eight photos were taken using Infinity Capture imaging software (Lumenera, Ottawa, Ontario, Canada). For each photo, five evenly shaped elliptical erythrocytes and corresponding nuclei major and minor axes were measured using ImageJ (version 1.8.0, National Institute of Health, Bethesda, Maryland, USA; n = 40 cells·fish^{-1}). Ellipsoid volume (V) and surface area (SA) were calculated using the following equations:

$$V = \frac{4}{3}\pi ab^2$$

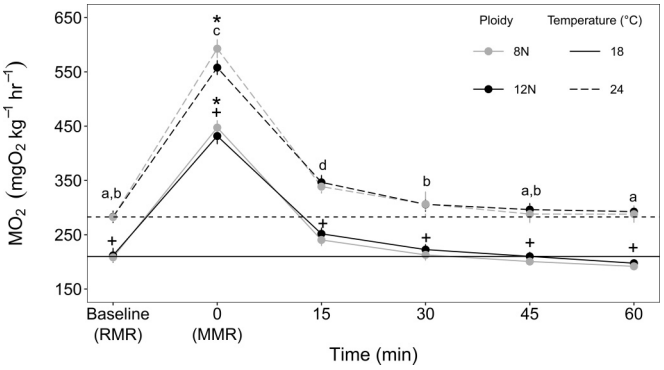
$$\mathrm{SA} = 4\pi\left[\frac{2(ab)^{1.6} + b^{3.2}}{3}\right]^{1/1.6}$$

where a = (major axis)/2 and b = (minor axis)/2.

Statistical analyses

All data were analyzed in R (http://www.R-project.org) using R Studio interface (version 3.5.1). Significance level (α = 0.05) was determined prior to statistical analysis. Before statistical tests were used, normality and heterogeneity of variance of residuals were visually inspected using a Q–Q plot and a fitted versus residuals plot, respectively. MO_2 data were analyzed using a two-way ANOVA with repeated measures, using ploidy and time as fixed factors. Chase–recovery data were analyzed with a two-way ANOVA with ploidy and time as fixed factors. Significant differences in surface area to volume ratio were analyzed with a two-tailed t test. A log transformation was used when the assumption of normality was not met. All reported values are mean ± 1 SE unless otherwise stated. Significant differences between treatment levels were determined using a Tukey's post hoc test ("lsmeans" package; Lenth 2016).

Fig. 1. Mass-specific metabolic rate (MO_2) of diploid (8N) and triploid (12N) white sturgeon at ambient (18 °C) and acute (2 h) elevated temperature (24 °C). Multiple measurements were taken over 18 h for baseline (RMR, routine metabolic rate) prior to a 3 min chase. Metabolic rate measurements were then taken immediately (MMR, maximum metabolic rate), 15, 30, 45, and 60 min following a 3 min chase protocol. A plus sign (+) denotes a significant effect of temperature (p < 0.05). An asterisk (*) identifies significant differences (p < 0.05) between ploidies for specific time points and temperatures. Different letters indicate significant differences (p < 0.05) between different sampling time points. Horizontal lines indicate the combined mean RMR for diploid and triploid white sturgeon at ambient (solid line) and elevated (dashed line) temperatures. Data points and error bars represent means ± 1 SE (n = 16 fish per ploidy and temperature).



Results

Morphometrics of diploid and triploid white sturgeon

There was no significant difference in weight between ploidies ($F_{[1,252]}$ = 1.10, p = 0.294) or temperature ($F_{[1,252]}$ = 3.06, p = 0.081). The mean and standard deviation of weights of 8N and 12N fish at ambient temperature were 12.7 ± 2.6 g (range: 7.8–18.7 g) and 13.0 ± 2.9 g (range: 6.6–21.3 g), respectively. The mean weights of 8N and 12N fish at the elevated temperature were 13.3 ± 2.5 g (range: 8.6–18.9 g) and 13.6 ± 2.4 g (8.3–19.4 g), respectively.

RMR, MMR, aerobic scope, and time to recovery of diploid and triploid white sturgeon

In general, metabolic rate was comparable between diploid and triploid white sturgeon except after chasing. Diploid and triploid sturgeon exhibited similar routine metabolic rates (RMR, $F_{[1,60]}$ = 0.15, p = 0.70; Fig. 1) but differed in maximum metabolic rates (MMR, $F_{[1,60]}$ = 5.32, p = 0.02; Fig. 1), with triploid sturgeon demonstrating a lower MMR. On average, absolute aerobic scope was 11% higher in diploid sturgeon at 18 °C and 18% higher in diploid sturgeon at 24 °C when compared with triploid sturgeon ($F_{[1,60]}$ = 7.96, p = 0.006; Fig. 2). Factorial aerobic scope was also significantly affected by ploidy ($F_{[1,60]}$ = 4.98, p = 0.03; Fig. 2). Despite differences in MMR, both diploid and triploid sturgeon recovered from exhaustive exercise within 30 min after chasing (i.e., metabolic rate was no longer statistically different from RMR).

For both diploid and triploid white sturgeon, an acute increase in water temperature increased RMR ($F_{[1,60]}$ = 43.71, p < 0.001; Fig. 1), MMR, ($F_{[1,60]}$ = 99.25, p < 0.001; Fig. 1), absolute aerobic scope ($F_{[1,60]}$ = 26.21, p < 0.001: Fig. 2), and time to recover from exhaus-

Fig. 2. Aerobic scope of diploid (8N) and triploid (12N) white sturgeon at ambient (18 °C) and acute (2 h) elevated temperature (24 °C). A plus sign (+) denotes a significant effect of temperature ($p < 0.05$). An asterisk (*) identifies significant differences ($p < 0.05$) between ploidies. Numbers in parentheses are the factorial aerobic scope for each treatment. Data points and error bars represent means ± 1 SE ($n = 16$ fish per ploidy and temperature).

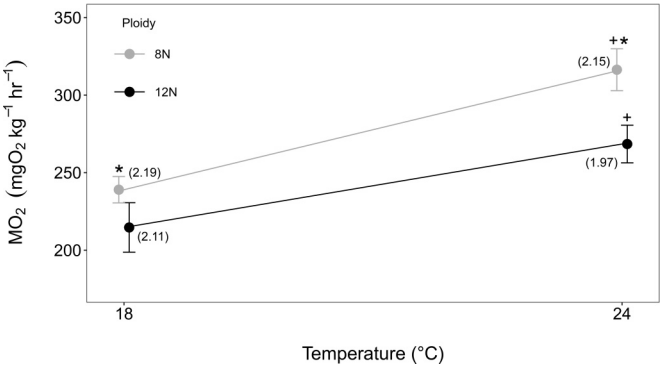
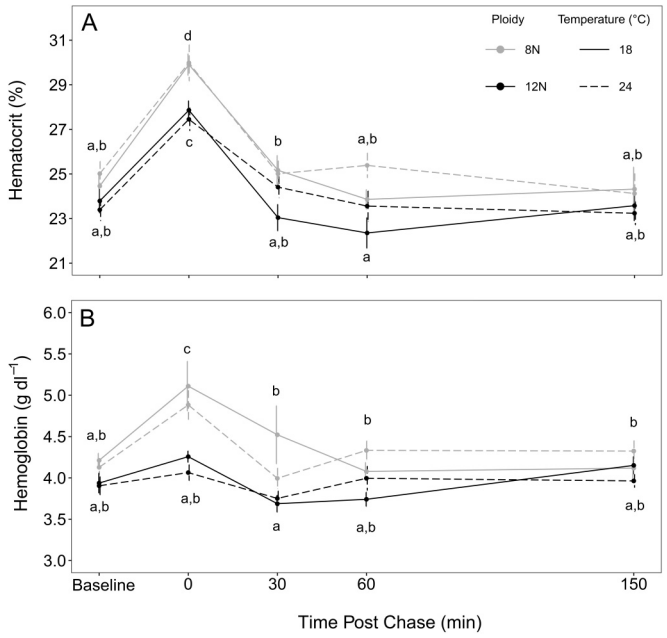


Fig. 3. Hematocrit (A) and total hemoglobin concentrations (B) of diploid (8N) and triploid (12N) white sturgeon at ambient (18 °C) and acute (2 h) elevated temperature (24 °C). Samplings occurred before (baseline), immediately (0 min), 30, 60, and 150 min following a 3 min chase protocol. Different letters indicate significant differences ($p < 0.05$) between ploidies across time points. Data points and error bars represent means ± 1 SE ($n = 16$ fish per ploidy, temperature, and time point).



tive exercise ($F_{[1,59]} = 95.24$, $p < 0.001$; Fig. 1); however, factorial aerobic scope was not significantly affected by temperature ($F_{[1,60]} = 0.42$, $p = 0.52$; Fig. 2). The Q_{10} values for RMR were 1.64 and 1.62 for diploid and triploid sturgeon, respectively, while the Q_{10} values for MMR were 1.62 and 1.54 for diploid and triploid sturgeon, respectively. Both diploid and triploid sturgeon responded similarly to an acute temperature increase, as there were no significant interactions between temperature and ploidy for RMR ($F_{[1,60]} = 0.001$, $p = 0.98$; Fig. 1), MMR ($F_{[1,60]} = 0.76$, $p = 0.39$; Fig. 1), absolute aerobic scope ($F_{[1,60]} = 0.85$, $p = 0.36$: Fig. 2), factorial aerobic scope ($F_{[1,60]} = 0.11$, $p = 0.74$; Fig. 2), or time to recover from exhaustive exercise ($F_{[1,59]} = 0.03$, $p = 0.85$; Fig. 1).

Hematological recovery from exhaustive exercise in diploid and triploid white sturgeon

Overall, diploid and triploid white sturgeon showed a similar trend in hematological responses to chasing, yet the magnitude of response immediately following chasing differed between ploidies. Although diploid sturgeon demonstrated a higher hematocrit at all time points compared with triploid sturgeon ($F_{[1,281]} = 26.94$, $p < 0.001$; Fig. 3A), both ploidies demonstrated a significant increase in hematocrit immediately after chasing and both returned to baseline levels within 30 min after chasing ($F_{[4,281]} = 47.79$, $p < 0.001$; Fig. 3A). Hemoglobin was also higher in diploid sturgeon ($F_{[1,299]} = 47.79$, $p < 0.001$; Fig. 3B); however, diploid sturgeon had a much greater magnitude of a response to chasing than triploid sturgeon, with a 19.8% change from baseline to immediately after chasing compared with a 6.1% in change in triploid sturgeon (significant ploidy by time interaction, $F_{[4,299]} = 3.25$, $p = 0.01$; Fig. 3B). Like hematocrit levels, hemoglobin concentration returned to baseline levels within 30 min after chasing in both diploid and triploid sturgeon. Although triploid white sturgeon had a lower magnitude of a response for hematocrit and hemoglobin following chasing, mean erythrocyte hemoglobin concentration (MEHC) was similar between ploidies ($F_{[1,281]} = 0.08$, $p = 0.78$) and was lower immediately following chasing ($F_{[4,281]} = 4.29$, $p = 0.002$). However, both ploidies responded similarly to chasing (interaction between ploidy and time was not significant; $F_{[4,281]} = 1.29$, $p = 0.27$). Moreover, temperature had no significant effect on MEHC ($F_{[1,281]} = 2.06$, $p = 0.15$).

Neither plasma lactate ($F_{[1,296]} = 0.04$, $p = 0.85$; Fig. 4A) nor plasma glucose ($F_{[1,296]} = 0.58$, $p = 0.45$; Fig. 4B) were affected by ploidy. Both diploid and triploid white sturgeon demonstrated elevated plasma lactate concentrations immediately after chasing that returned to baseline levels within 150 min after chasing ($F_{[4,296]} = 62.31$, $p < 0.001$; Fig. 4A). Plasma glucose levels significantly increased immediately after chasing and remained elevated 150 min after chasing, in both ploidies ($F_{[4,296]} = 23.71$, $p < 0.001$; Fig. 4B). Additionally, acute exposure to elevated water temperature did not impact plasma lactate ($F_{[1,296]} = 0.29$, $p = 0.59$; Fig. 4A) or plasma glucose ($F_{[1,296]} = 3.44$, $p = 0.06$; Fig. 4B) levels for either ploidy.

Erythrocyte and nuclear surface area to volume ratios in diploid and triploid white sturgeon

Overall, triploid white sturgeon had larger erythrocytes and nuclei when compared with diploid sturgeon. Calculated erythrocyte and nuclear volumes were 50.6% and 40.0% larger, respectively, in triploid sturgeon compared with diploid sturgeon. On average, erythrocyte surface area to volume ratios from diploid sturgeon were 1.14 times larger than those from triploid sturgeon ($F_{[1,58]} = 331.7$, $p < 0.001$; Fig. 5A). Additionally, nuclei surface area to volume ratios were 1.11 times larger in diploid compared with triploid sturgeon ($F_{[1,58]} = 226$, $p < 0.001$; Fig. 5B).

Discussion

With growing evidence that triploid salmonids underperform in suboptimal conditions and a lack of information regarding triploid sturgeon physiology, we sought to examine metabolic and hematological recovery from exhaustive exercise at two temperatures in diploid and triploid white sturgeon. Overall, both ploidies recovered metabolically and hematologically from exhaustive exercise within the same amount of time; however, the main difference found between ploidies was a difference in the magnitude of response immediately following exercise. Triploid white sturgeon had a reduced maximum metabolic rate and hematocrit, as well as a lower hemoglobin concentration compared with diploid white sturgeon immediately following exhaustive exercise. Determination of cellular and nuclear dimensions of

Fig. 4. Plasma lactate (A) and plasma glucose (B) of diploid (8N) and triploid (12N) white sturgeon at ambient (18 °C) and acute (2 h) elevated temperature (24 °C). Samplings occurred before (baseline), immediately (0 min), 30, 60, and 150 min following a 3 min chase protocol. Different letters indicate significant differences ($p < 0.05$) between time points (ploidies were pooled since there was no significant effect of ploidy on plasma lactate or glucose). Data points and error bars represent means ± 1 SE ($n = 16$ fish per ploidy, temperature, and time point).

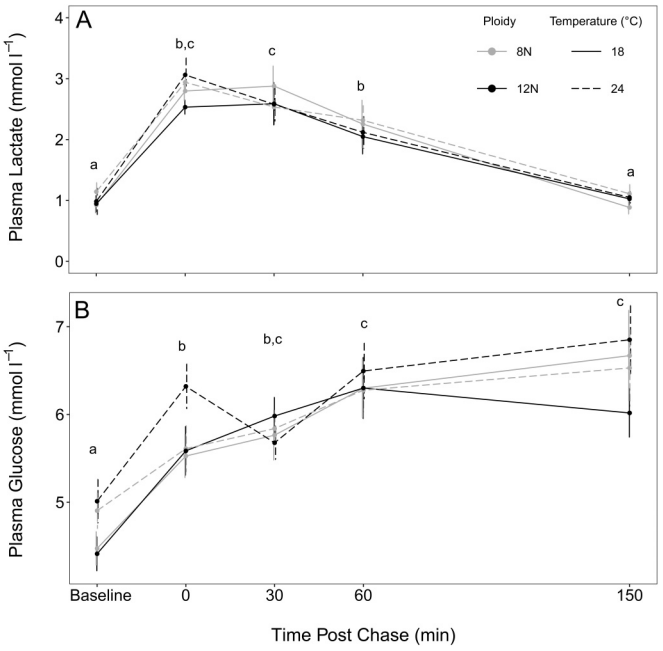
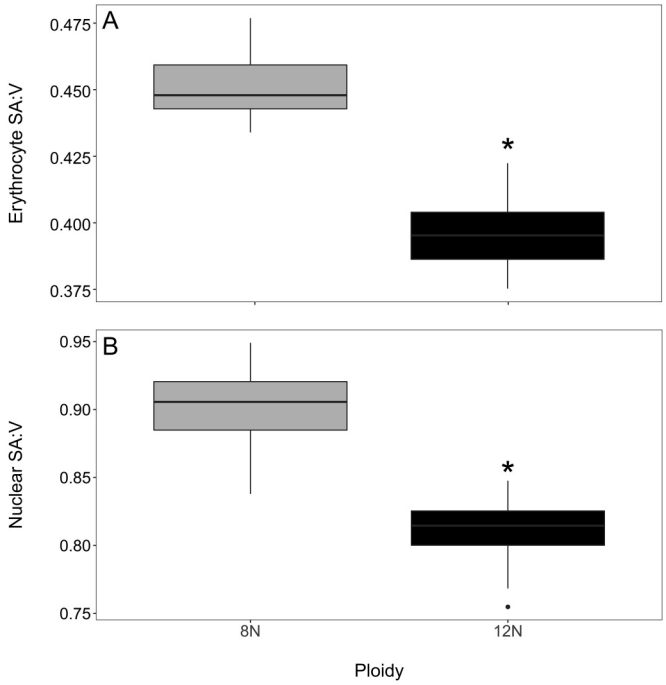


Fig. 5. Surface area to volume ratios (SA:*V*) of diploid (8N) and triploid (12N) white sturgeon erythrocytes (A) and nuclei (B). An asterisk (*) identifies significant differences ($p < 0.05$) between ploidies ($n = 30$ fish per ploidy).



diploid and triploid white sturgeon erythrocytes provided evidence of the reduction in surface area to volume ratios (SA:*V*) in triploid sturgeon erythrocytes and nuclei. The lower SA:*V* in triploid sturgeon erythrocytes can directly impact oxygen diffusion rates, while a lower hemoglobin concentration implies a lower oxygen carrying capacity immediately following chasing. Together, SA:*V* and hemoglobin may account, in part, for the reduced maximum metabolic capacity following exercise in triploid white sturgeon.

Resting and maximum metabolic rates, aerobic scope, and metabolic recovery from exhaustive exercise in diploid and triploid white sturgeon

At both ambient and acute elevated temperature exposure, diploid and triploid white sturgeon were similar in RMR but differed in their MMR (Fig. 1), and, therefore, aerobic scope. Triploid white sturgeon demonstrated lower aerobic scope, suggesting a reduced capacity to fuel biological processes such as growth, reproduction, and activity (Fig. 2). Many researchers have reported metabolic differences in standard metabolic rate (SMR), MMR, and aerobic scope (AS) in diploid and triploid salmonids. Studies have found a lower metabolic rate following exercise in triploid brook trout (Hyndman et al. 2003*b*), a reduced factorial AS in triploid brown trout at elevated temperatures (Altimiras et al. 2002), higher estimated SMR in triploid brook char (O'Donnell et al. 2017), and lower oxygen consumption in triploid Atlantic salmon (*Salmo salar*) at higher temperatures (Sambraus et al. 2017). In contrast, Bowden et al. (2018) reported similar RMR in diploid and triploid Atlantic salmon after 7 weeks of acclimation, Lijalad and Powell (2009) showed no differences in diploid and triploid RMR or MMR, and Scott et al. (2015) found no differences in RMR in diploid and triploid rainbow trout (*Oncorhynchus mykiss*). In our study, AS was 10%–20% lower in triploid white sturgeon, both at ambient and after acute exposure to elevated temperatures. While most studies with salmonids focused on the effects of warm temperature acclimation, our study demonstrated that with and without acute thermal stress, triploid white sturgeon had a reduced aerobic capacity, which could leave them with less energy available for processes beyond maintenance, such as growth and reproduction. The difference in MMR in white sturgeon may be due, in part, to differences in cellular aerobic metabolism. Previous studies in our group have shown that compared with diploid white sturgeon, triploid sturgeon have a reduced ability to upregulate citrate synthase after exposure to acute stress (Leal et al. 2019). The lower MMR in triploid white sturgeon may be due to a hindered ability to make short-term adjustments to metabolic enzymes to adjust cellular aerobic metabolism.

Alternatively, since diploid and triploid white sturgeon exhibited a similar RMR, the difference in MMR might imply that less energy is required to deal with the chasing protocol for triploid sturgeon, suggesting a more efficient physiology when compared with diploid sturgeon. However, this would suggest that some plasticity exists in maximum aerobic capacity. In European perch (*Perca fluviatilis*), there is evidence to suggest that resting aerobic capacity is more thermally plastic than maximum aerobic capacity (Sandblom et al. 2016). Thus, in fish, it appears that resting metabolic rate is more plastic than MMR; therefore, we would expect a more efficient physiology to demonstrate a lower resting metabolic rate rather than a lower MMR. Moreover, if triploid sturgeon had a more efficient physiology, they should be capable of being chased longer before becoming exhausted; however, this was not the case in our pilot study (see description of chasing protocol in the Materials and methods section). Additionally, given that the maximum cellular aerobic capacity was reduced in triploid white sturgeon exposed to acute water reduction stress (Leal et al. 2019), it appears that triploid white sturgeon are limited in their capacity rather than more efficient. In either case, AS is an indication of the energy an organism has available for processes beyond maintenance, including stressors like chasing. The results

from our study provide evidence that triploid white sturgeon have a reduced AS compared with diploid sturgeon.

Despite differences in MMR and AS, both diploid and triploid white sturgeon exhibited similar recovery kinetics from exhaustive exercise (Fig. 1). Likewise, diploid and triploid brook trout exhibited different metabolic rates only immediately after exercise, but demonstrated similar metabolic rates before exercise and similar times to recover from exercise (Hyndman et al. 2003*b*). Metabolic rates in the current study are consistent with previously published metabolic rates of juvenile white sturgeon (Crocker and Cech 1997). The twofold increase in metabolic rate after chasing and recovery of metabolic rate are consistent with studies of exhaustive exercise in shortnose sturgeon (Zhang and Kieffer 2017). Both ploidies recovered from exhaustive exercise within 30 min at ambient temperature and within 45 min after acute elevated temperature exposure. Our results suggest triploid physiology inhibits MMR but does not impact the process of recovery of aerobic metabolism after exhaustive exercise, as both ploidies recovered within the same time frame.

Hematological recovery from exercise in diploid and triploid white sturgeon

In triploid white sturgeon, both hematocrit and hemoglobin were consistently lower than in diploid white sturgeon across temperatures before, immediately following, and during recovery from exhaustive exercise (Fig. 3). These inherent differences in hematology have previously been recorded in triploid white sturgeon (Leal et al. 2019). Moreover, before and immediately after chasing, triploid white sturgeon showed a reduced magnitude of change in hematocrit and hemoglobin concentrations (Fig. 3), demonstrating that triploid white sturgeon did not respond similarly to chasing when compared with diploid white sturgeon. Our findings differ from studies in salmonids, which reported no difference in diploid and triploid hemoglobin following exhaustive exercise (Hyndman et al. 2003*a*); however, Hyndman and colleagues also reported no differences in hemoglobin between the ploidies prior to chasing. Additionally, Beyea et al. (2005) showed that diploid and triploid shortnose sturgeon did not show differences in total hemoglobin, as triploid sturgeon had more hemoglobin per cell. Moreover, given that MEHC was similar between ploidies yet total hemoglobin was lower, especially immediately after chasing, unlike other triploid fishes that are capable of compensating for fewer erythrocytes with more hemoglobin per cell, it appears triploid white sturgeon cannot fully compensate for having fewer erythrocytes.

The muted response of hematocrit and especially hemoglobin to chasing in triploid white sturgeon may help explain differences in MMR. The greatest difference between diploid and triploid white sturgeon hematocrit and hemoglobin levels was immediately following chasing. MEHC was similar between ploidies and was lower only immediately after chasing, suggesting a brief period of erythrocyte swelling following exhaustive exercise. Previous work with triploid sturgeon has also demonstrated similar MEHC between diploid and triploid sturgeon (Beyea et al. 2005; Leal et al. 2019). Since this response was similar between ploidies, the adrenergic response that causes cell swelling (Jensen 2004) does not vary between diploid and triploid white sturgeon. It is more likely that either triploid sturgeon have fewer erythrocytes stored or they are not capable of releasing a comparable number of erythrocytes to increase hemoglobin to the same degree as diploid sturgeon. More research is required to determine the reason behind the lower hemoglobin exhibited by triploid white sturgeon following exercise. In either case, the total hemoglobin concentration remains lower in triploid white sturgeon, which could result in a lower oxygen carry capacity especially in energetically demanding scenarios, such as exhaustive exercise.

Despite differences in whole animal aerobic metabolism, diploid and triploid white sturgeon had similar plasma metabolite concentrations before and during recovery from exercise, suggesting that a reduction in SA:*V* does not impair transport of plasma metabolites. Plasma lactate and glucose concentrations did not differ between ploidies or across temperatures before, immediately after, or during recovery from exhaustive exercise (Fig. 4). Our findings are consistent with Hyndman et al. (2003*a*), who found no difference before or immediately following exercise in levels of plasma lactate or glucose in diploid and triploid brook trout. Additionally, Beyea et al. (2005) found no difference in diploid and triploid plasma glucose concentrations before or after handling and confinement stress. Unlike hematocrit and hemoglobin, plasma lactate and glucose did not show a difference in magnitude of response to exhaustive exercise between diploid and triploid white sturgeon. Since lactate and glucose rely on transporter proteins (Hertz and Dienel 2005; McCall 2019), the limitations of surface area on passive diffusion may not apply to the transport of these metabolites in triploid white sturgeon. Baseline plasma lactate and glucose measurements were consistent with those previously reported in diploid and triploid white sturgeon (Leal et al. 2019). It is important to note, however, that glucose levels did not return to resting levels within measured time points of the experiment (i.e., 150 min). It appears that sturgeon may need more than 150 min to recover from exhaustive exercise, as both diploid and triploid shortnose sturgeon did not show any change in plasma glucose concentrations up to 6 h after 15 min of chasing (Beyea et al. 2005). More research is needed to determine whether the final time for plasma glucose concentrations to return to baseline after exercise would be different between diploid and triploid white sturgeon.

Surface area to volume ratios of diploid and triploid white sturgeon erythrocytes and nuclei

Triploid white sturgeon had larger nuclei and erythrocytes, resulting in lower surface area to volume ratios, compared with diploid sturgeon. Previous studies have shown triploid white sturgeon have generally larger erythrocytes compared with diploid sturgeon (Fiske et al. 2019). Erythrocyte volume in triploid white sturgeon was ~50% larger, while nuclear volume was ~40% larger, which falls in line with predictions based on genome size of triploid fishes (Benfey 1999). Since volume increases at a faster rate than surface area with an increase in axis length, the larger erythrocyte and nuclear volumes in triploid white sturgeon will reduce SA:*V* (Fig. 5). If all dimensions of a cell grow proportionally with an increasing genome, we would predict the major and minor axes each to be 16.7% larger (50% larger divided by three dimensions) and therefore the SA:*V* to be 16.7% larger in diploid fish than in triploid fish since volume is one dimension larger than surface area. We found that diploid erythrocytes, on average, had a 14% larger SA:*V*, following within the range of predicted differences in diploid and triploid cellular SA:*V*.

It is interesting to note that the percent difference in erythrocyte SA:*V* between diploid and triploid sturgeon (14%) is similar to the percent difference in absolute AS (11%–18%). A lower SA:*V* in triploid white sturgeon can result in a reduction in diffusion rates because the rate of diffusion is directly correlated to surface area (i.e., Fick's equation; Marx et al. 1960). This could be especially important for molecules that rely heavily on passive diffusion, such as oxygen (Marx et al. 1960), which could then impact diffusion-limited processes, such as cellular metabolism (Nathan and Singer 1999). In fact, studies have demonstrated an inversely proportional relationship between genome size and SMR (Maciak et al. 2011) and between cell size and RMR in multiple fish species (Luo et al. 2015). To our knowledge, there has been no work to assess the link between genome or cell size and MMR in ectotherms; however, in birds, smaller genomes have been linked to larger heart indices (ratio of heart to body size; Wright et al. 2014), which is correlated with a higher MMR and AS (Bishop 1999). While no differences were found between diploid and triploid

white sturgeon RMR in our study, the differences in SA:*V* may place limitations on oxygen diffusion and hinder MMR in triploid white sturgeon.

Alternatively, the difference in absolute and factorial AS between diploid and triploid white sturgeon may be more related to elevated temperature rather than to cellular dimensions. The difference in AS between diploid and triploid sturgeon was larger when exposed to acute elevated temperatures (although the interaction between ploidy and temperature was not significant), which may indicate a lower tolerance to acute temperature change in triploid sturgeon. Several studies have demonstrated poorer performance of triploid fishes when exposed to elevated temperatures (see Maxime 2008 for a review). Previous studies have shown a slightly reduced stress response in triploid shortnose sturgeon (Beyea et al. 2005). Thus, the lower MMR seen in triploid white sturgeon, especially after acute elevated temperature exposure, may be a reduction in the stress response to elevated temperature.

While AS was significantly lower in triploid white sturgeon, whether this translates to a biological difference in how energy is allocated to different processes in diploid and triploid white sturgeon is unclear. In the current study, both ploidies were capable of tolerating the chasing and elevated temperature stressors. Additionally, in general, diploid and triploid white sturgeon demonstrated similar primary and secondary responses to acute water reduction and handling stressors (Leal et al. 2019). Therefore, triploid sturgeon may have a compensatory method for dealing with a reduced SA:*V*. Differences in mitochondrial density or location relative to cell surface could be one mechanism accounting for the relatively small difference in AS between ploidies. Previous studies have shown no differences in citrate synthase activity in diploid and triploid white sturgeon (Leal et al. 2019), an indication of mitochondrial volume; however, the localization of mitochondria inside a cell is a heavily regulated process (MacAskill and Kittler 2010). More research is needed to determine if mitochondrial localization is altered in triploid white sturgeon such that mitochondria are located closer to the plasma membrane to reduce the distance oxygen needs to travel by diffusion.

Conclusions

The results of our study indicate that at both ambient temperature and following acute elevated temperature exposure, diploid and triploid white sturgeon differed in their physiological response to exhaustive exercise, yet were similar in recovery kinetics from exhaustive exercise. Triploid white sturgeon exhibited a reduced aerobic scope (AS), hematocrit and hemoglobin immediately after chasing, compared with diploid white sturgeon, suggesting a lower oxygen carrying capacity and less energy for biological processes beyond maintenance in energetically demanding environments. This difference may be linked to the lower surface to volume ratio found in triploid white sturgeon. Together, these limitations in triploid physiology may not hinder maintenance and resting state of triploid white sturgeon but may inhibit the maximum aerobic metabolic capacity when metabolism must acutely increase. The reduction in AS found in triploid white sturgeon could have implications for other biological processes. In environments where different stressors could occur, such as aquaculture facilities, energy is devoted to coping with stressors (Davis 2006). Having a reduced AS could leave triploid white sturgeon with less energy to cope with stress and ultimately influence growth. Triploid white sturgeon are fertile, and therefore a lower AS may mean triploid sturgeon allocate less energy to reproduction, somatic growth, and (or) activity compared with diploid sturgeon. Previous work in our lab suggests that triploid sturgeon may have lower specific growth rates, especially when exposed to elevated temperatures (Leal et al. 2019). The allocation of energy to various biological processes is still understudied in many fish species, including sturgeon. Because the difference in AS was relatively small, more research is needed to determine whether there are whole organism consequences of a reduced AS in triploid white sturgeon, as white sturgeon farms and conservation hatcheries implement management strategies for spontaneous autopolyploidy in white sturgeon. Although triploid fishes may provide advantages in an aquaculture setting (potential for higher growth rates), given the increasing evidence that triploid salmonids underperform in suboptimal environments (see Benfey 1999 and Maxime 2008 for reviews), a better understanding of limitations to aerobic metabolism and energy allocation between ploidies will not only benefit cultured sturgeon, but cultured salmonids as well.

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