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Reduced growth may be linked to lower aerobic scope in juvenile triploid white sturgeon (*Acipenser transmontanus*)

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ABSTRACT

Previous studies have provided evidence of a reduced aerobic metabolic capacity, both at the cellular (metabolic enzyme activity) and the whole organism (aerobic scope) level in juvenile triploid white sturgeon, compared to diploid siblings. The downstream costs of this reduced metabolic capacity are still unclear, yet a lower aerobic scope suggests triploid white sturgeon likely have less energy to allocate to biological processes like growth and development. We conducted a 15-week growth trial to assess energy allocation to somatic growth in 2-month-old diploid and triploid white sturgeon. Spontaneous swimming activity, hepatosomatic index, condition factor, and deformities were also measured throughout the growth trial as indices of energy allocation to activity and fish condition. In general, our results indicate that triploid white sturgeon may have less energy available for processes beyond basal maintenance. This could be linked to a reduced overall performance as evidenced by lower weights and more deformities when compared with their diploid counterparts. However, many indices were still mostly unaffected by triploidy (condition factor, hepatosomatic index, and swimming activity). Whether this lower growth performance seen in juvenile triploid white sturgeon continues through sub-adulthood, puberty and final maturation requires further long-term studies.

1. Introduction

Sturgeon aquaculture has been an emerging industry since the 1960s but has grown significantly in the last 20 years (Bronzi et al., 2011). The development of sturgeon aquaculture as an alternative to exploiting wild sturgeon fisheries for the production of meat and caviar has alleviated pressure on declining sturgeon populations (Bronzi et al., 2011). White sturgeon (*Acipenser transmontanus*), was the first sturgeon species to be commercially cultured in North America, with a completely closed reproductive cycle and F3+ generations of captive broodstock (Van Eenennaam et al., 2004). While the biology of white sturgeon was well studied in the development of culture practices, there is still information to be learned about how polyploidy in white sturgeon affects various aspects of their physiology.

In the diploid state, white sturgeon have eight copies of each chromosome (8 N), while triploid white sturgeon contain twelve copies of each chromosome (12N) (Drauch Schreier et al., 2011). Triploid fishes (i.e. those that contain an extra set of chromosomes) have been utilized in salmonid aquaculture due to their sterility and, thus, have a reduced risk of genetically influencing wild stocks and potential for higher growth rates (Benfey, 1999). Unlike triploid salmonids, triploid white sturgeon are reproductively fertile, producing 6 N gametes (Drauch Schreier et al., 2011). Since triploid gametes have 50% more genetic material than diploid gametes, there is potential for triploid eggs to be larger than diploid eggs, which is a potential benefit to the caviar industry.

To better understand the role for triploid white sturgeon in the caviar industry, it is important to understand any costs associated with triploidy. Poor physiological performance (i.e. reduced growth, mortalities and disease resistance) is often reported in triploid salmonids in sub-optimal environments (reviewed by Benfey, 1999; Maxime, 2008). Moreover, studies on the growth in triploid fish has yielded mixed results, with some triploid fish exhibiting lower, equal, or higher growth performance compared with their diploid counterparts (Galbreath et al., 1994; McGeachy et al., 1995; Carter et al., 1994). In general, when reared in optimal conditions, triploid salmonids demonstrate similar growth rates to diploid salmonids during early life stages, but superior growth rates after sexual maturation to their diploid counterparts (Piferrer et al., 2009). Yet, there is clear evidence that triploid salmonid growth is reduced compared to diploid fish when reared in high

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temperatures (Ojolick et al., 1995; Hansen et al., 2015; Sambraus et al., 2017). The cause of this discrepancy in performance between ploidies at high temperatures is not yet clear, but a reduced aerobic scope for activity may leave less energy available to cope with stressors and/or to grow (Fraser et al., 2012). Thus, understanding the aerobic scope and energy allocation in triploid fishes requires further study, especially in sturgeon.

Energy allocation to all biological processes is difficult to quantify; however, specific processes that require a larger proportion of energy (i.e. growth) or that are regulated to conserve energy (i.e. storage and activity) can be assessed to provide insight into the distribution of available energy beyond maintenance (i.e. energy for aerobic scope). White sturgeon exhibit high growth rates, especially in early developmental stages, and allocate a moderate amount of metabolizable energy to growth (Detlaf, 1993; Cui et al., 1996). Condition factor and hepatosomatic index provide insight into the overall state of the fish and correlate to energy storage (Williams, 2000; Schloesser and Fabrizio, 2017). Decreasing swimming activity is one mechanism for decreasing metabolic demand when available energy is reduced (Detlaf, 1993). White sturgeon reduced swimming activity when metabolic rate was suppressed due to exposure to hypoxia (Crocker and Cech, 1997). Therefore, growth, condition, and activity are processes where we might be able to see changes in energy allocation to account for a lower aerobic scope.

To date, several studies comparing the physiology of diploid and triploid white sturgeon have demonstrated that the primary distinction between the two ploidies is attributed to differences in energy metabolism. Triploid white sturgeon were found to have a lower surface area to volume ratio (SA:V) in both erythrocytes and nuclei; this difference in SA:V may limit diffusion mediated processes such as aerobic metabolism (Leal et al., 2020). For example, a reduction in SA:V may hinder diffusion mediated processes, like oxygen diffusion, which would reduce the maximum aerobic capacity (maximum metabolic rate) and, thus, aerobic scope. Furthermore, comparisons of a cellular aerobic capacity (i.e. citrate synthase enzyme activity) and whole organism metabolism suggest that triploid white sturgeon are limited in their maximum aerobic metabolic capacity (Leal et al., 2019; Leal et al., 2020). It is still unclear, however, if these differences in aerobic metabolism affect energy allocation to different processes like growth, condition, and activity. Preliminary evidence from a previous study suggests that triploid white sturgeon exhibit reduced growth rates compared to diploid white sturgeon, and especially when exposed to chronically elevated temperatures (Leal et al., 2019). Thus, in order to understand the effect of triploidy on energy metabolism in white sturgeon, we conducted a 15-week growth trial while also assessing resting metabolic rate, maximum metabolic rate following exhaustive chase and different biological processes (e.g. spontaneous swimming activity, condition factor, hepatosomatic index, and physical deformities) as indices of aerobic scope functions (e.g. activity and development). Based on previous findings in diploid and triploid white sturgeon, we predict triploid sturgeon will have a reduced aerobic scope and will, consequently allocate less energy to growth, storage, and activity (i.e. lower growth indices, condition factor, hepatosomatic index, and swimming activity) in order to balance the reduced energy supply.

2. Materials and methods

2.1. Fish source, triploid induction, and husbandry

Ovulated eggs were collected from three different female domesticated broodstock from a Northern California farm. Eggs from each female were fertilized separately, using pooled milt from three male broodstock. Spawning induction, fertilization and egg de-adhesion followed standard protocols (Van Eenennaam et al., 2004). To induce triploidy, fertilized eggs were heat shocked following procedures similar to Van Eenennaam et al. (1996). Briefly, 12–16 min post-fertilization, eggs were heat shocked at 32 °C for 3.5 min. At the designated time all the eggs being silted were poured into a fine-mesh dip net and placed into a 10 gal aquarium with air stones and fullers earth at 32 °C, for 3.5 min, then lifted out of the aquarium, placed back into the silting bowl (maintained at 15 ± 0.5 °C), and completed a total of 1 h of silting. Control eggs from each female (300 ml) were not exposed to a heat shock and were fertilized and silted under the standard farm protocol.

After fertilization and de-adhesion, eggs were transported in oxygen filled bags in an ice chest (15 ± 0.5 °C) to the Putah Creek Hatchery Facility at the University of California, Davis, CA, USA. All egg treatments were incubated in separate McDonald jars in a flow-through hatchery system (15.5 ± 0.5 °C). Fertility rates were between 70 and 98% and neurulation rates were between 59 and 85% for heat shocked and control groups for all three females. After hatch, larvae were transported to the Center for Aquatic Biology and Aquaculture facilities at the University of California, Davis. Larvae from each heat shock and control group were kept separate and were maintained in six separate circular fiberglass tanks (122 cm diameter, 650 l) at 18–19 °C.

At approximately 5–8 days post-hatch, 150 larvae from each tank were analyzed for ploidy using a coulter counter (Fiske et al., 2019). All control groups contained 100% 8 N larvae, while the heat shock group contained 95.3–100% 12 N larvae. One week after hatching, 1000 larvae from each control group were pooled into one stock tank, while 1000 larvae from each heat shock group were pooled into a different stock tank. Following yolk-sac depletion, fish experienced a typical feeding regime as described in Leal et al. (2018) until experimentation at two-months post hatch.

2.2. Growth trial

Two-months after hatching, 1050 diploid (8 N) and 1050 triploid (12 N) white sturgeon juveniles were randomly distributed from stock tanks to 14 circular fiberglass tanks (94 cm inner diameter, 50.3 ± 1.2 l volume) supplied with degassed, flow-through, well water at a flow rate of 11.9 ± 0.4 l/min (n = 150 fish/tank and 7 tanks/ploidy). Fish were fed continuously using 24-h belt feeders that were filled once daily. Each day, temperature and dissolved oxygen was recorded, mortalities were removed from each tank, fish waste was removed by lifting the standpipe for 10 s, and excess feed on the feeder was brushed into the tank. Temperature and dissolved oxygen were maintained at 18.7 ± 0.4 °C and 8.0 ± 0.4 mg O_2 l^{-1}, respectively.

Feed rates were based on empirically validated optimum feeding rate models established for white sturgeon weighing 0.05–800 g (Lee et al., 2014). Both 8 N and 12 N fish were fed the same rate, until week 9 of experimentation when weights of the two ploidies were significantly different (see Supplemental Materials for feed rates). Triploid white sturgeon had lower weights and were, therefore, fed at a higher rate compared to diploid white sturgeon in accordance with the model used (Lee et al., 2014). At the start of the experiment (day 0), 100 fish per stock tank were euthanized in a lethal dose of sodium bicarbonate buffered tricaine methanesulfonate (MS-222, 500 mg/l) and body weight, total length, liver weight, and deformities were all recorded. Blood was sampled and used to verify ploidy using a Coulter counter (Fiske et al., 2019). Any 8 N fish sampled from a designated 12 N tank was removed from analysis. Every three weeks, fish were randomly sampled from each replicate tank and sampled as described above. At week 3, 30 fish from each tank were sampled, at weeks 6 and 9, 25 fish were sampled from each tank, and at weeks 12 and 15, 20 fish from each tank were sampled. The number of fish sampled each week was based on maintaining similar total fish biomass across the trial. Feed rates were adjusted every three weeks based on mean weight values for each ploidy (see Supplemental Materials for feed rates).

Feed efficiency (FE), specific growth rate (SGR), hepatosomatic index (HSI), and condition factor (K) were calculated using the following equations:

$$FE\ (\%) = \frac{Total\ weight\ gained\ per\ tank\ (g)}{Total\ weight\ of\ feed\ per\ tank\ (g)}\ x\ 100$$

$$SGR\ \left(\%day^{-1}\right) = \frac{ln(Final\ weight\ (g)\ ) - ln(Initial\ weight\ (g)\ )}{Time\ (day)} x\ 100$$

$$HSI\ (\%) = \frac{Liver\ weight\ (g)}{Fish\ weight\ (g)}\ x\ 100$$

$$K = \frac{Fish\ weight\ (g)}{Total\ length\ (mm)^3}\ x10000$$

2.3. Aerobic scope

To determine whole organism aerobic scope in 8 N and 12 N white sturgeon, routine metabolic rate (RMR) using intermittent-flow respirometry and maximum metabolic rate (MMR) using manual exhaustive chasing were assessed at during weeks 1, 6, and 15. Experimental protocols during week 1 were similar to those described in Leal et al. (2020). Briefly, intermittent flow respirometry was used to measure oxygen consumption rates in glass respirometers (mean volume 0.499 l), submerged in a water bath (circular fiberglass tank, 96 cm diameter, 142 l) with a UV filter to minimize microbial growth. The water bath was supplied with the same degassed well water as the tanks used for the growth trial (mean temperature 18.7 ± 0.4 °C). Mass specific metabolic rate (MO_2) was calculated using the following equation:

$$\text{Metabolic rate}\ \left(mgO_2\ kg^{-1}\ h^{-1}\right) = (\Delta O_2 \times V)/(M \times T)$$

where ΔO_2 is the change in oxygen concentration over the measurement period (in mgO_2/l), 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).

For week 1, respirometry trials took place over four days with eight fish (four fish/ploidy) each day. Ploidy was verified after experimentation in respirometers. In some cases, a sturgeon from a 12 N tank was verified to be 8 N and was removed from analysis but this was never more than one fish per ploidy at any given sampling time. Therefore, even though four fish per ploidy per day were used, the sample size was sometimes smaller (n = 15–16 fish/ploidy).

Previous studies have shown that postprandial metabolic rate remains elevated for up to 21 h following a meal in Siberian sturgeon (Dabrowski et al., 1987). Thus, to eliminate variations in metabolic rate due to postprandial changes in oxygen consumption, fish were fasted 24 h prior to experimentation. On each trial day, individual fish were placed in respirometers and MO_2 was measured for a 22 h resting period. During this resting period, oxygen consumption was measured every 19 min (12-min flush, 7-min measure). During the measurement period, water in the respirometers was recirculated using individual submersible pumps connected to each respirometer via tubing. The following morning after the resting period, fish were manually chased by hand for three minutes in an aerated bucket (28 cm diameter, 20 cm height) submerged in a water bath. After chasing, fish were subjected to a 15 s air exposure in a fine mesh net, then immediately returned to their respective respirometers. Oxygen consumption was measured for three minutes immediately after chasing. Fish were then euthanized with a lethal dose of MS-222; lengths and weights were recorded, and blood was sample for ploidy verification.

Week 6 and 15 respirometry trials were conducted similarly to week 1, except for the size of the respirometers, the chase time, and the timing of measurement and flush cycles. During week 6, larger glass jars (mean volume 1.94 l) were used to accommodate larger fish. During week 15, cylindrical, acrylic flow-through respirometers (mean volume 4.01 l) were used. Respirometers were fitted with a flat piece of acrylic attached with aquarium sealant approximately ¼ from the bottom, allowing the sturgeon to rest comfortably. The respirometers were placed in an aerated water bath (rectangular fiberglass tank, 157 cm length, 61 cm width, 37 cm height) with a UV filter. For both sets of trials, during the 22 h resting period, oxygen consumption was measured every 36 min (30-min flush, 6-min measure) and measured for three minutes after chasing. This flush and measure time was based on ensuring the oxygen level of water never went below 80% air saturation during the measurement period and that oxygen levels remain consistent for at least ten minutes during the flush period. Fish were chased manually for ten minutes. For week 6 trials, like week 1 trials, respirometry trials took place over four days with eight fish (four fish/ploidy) each day. Any sturgeon from 12 N designated tanks that were found to be 8 N were removed from analysis (n = 15–16/ploidy). During week 15, respirometry trials took place over five consecutive days with six fish (three fish/ploidy) per day and 8 N sturgeon from 12 N tanks were removed from analysis (n = 14–15 fish per ploidy). For all trials, before experimentation, four fish from each ploidy were chased by hand until exhaustion, which was characterized by the fish no longer responding to chasing and loss of equilibrium (Leal et al., 2020). Chasing time was determined by the time in minutes that was sufficient to exhaust a total of eight fish (four fish/ploidy) for each week. For all three weeks, there was no difference in time to exhaustion between 8 N and 12 N sturgeon. RMR was calculated as twentieth quantile of the measurements taken during the 22 h resting period. MMR was the MO_2 immediately following chasing. Factorial aerobic scope was calculated as the ratio tsy of MMR to RMR, while absolute aerobic scope was calculated as the difference in MMR and RMR.

2.4. Spontaneous swimming activity

To compare baseline swimming activity of 8 N and 12 N white sturgeon, video recordings of individual fish were taken during weeks 1, 6, and 15. For all weeks, video trials took place in a water bath (circular fiberglass tank, 122 cm diameter) to maintain temperature throughout the duration of the video trial. The water bath was supplied with the same degassed well water at the growth trial tanks (mean temperature 18.7 ± 0.4 °C), filled with approximately 70, 115, and 210 l of water for weeks 1, 6, and 15 respectively. Two trials on two consecutive days were conducted with four 8 N and four 12 N fish per trial between 7:30 and 10:00 AM (n = 15–16 fish/ploidy for each week). For each trial, fish were randomly placed in a single bucket or trash can with lids containing a drilled hole to allow cameras (Dragon Touch, Frederick, MD, USA) to rest on the lid.

During week 1, individual fish were placed in 5-gal buckets (26.67 cm diameter), filled with water 5.5 cm from the bottom of the bucket (3.07 l). During week 6, individual fish were placed in 20-gal trash cans (48.26 cm diameter), filled with water 10 cm from the bottom of the trash can (12.17 l). During week 15, individual fish were placed in 55-gal trash cans (66.04 cm diameter), filled with water 18 cm from the bottom of the bucket (40.22 l). For all weeks, in order to maintain consistent filming conditions, a line was marked on the inside of the bucket/ trash can to indicate a fill line for water. Additionally, a line was placed on the outside of the lid and bucket/ trash can to indicate where to place the lid, such that the lid and camera were always placed in the same location for each video.

After the 50-min video trial, fish were removed from their bucket or trash can and immediately euthanized in a lethal dose of MS-222. Body weight and total length were recorded, and blood samples were taken to verify ploidy. Any 8 N fish that were sampled from a designated 12 N tank were removed from analysis.

Videos were analyzed using EthoVision behavior software with the Social Interaction Module (v.13, Noldus Information Technology). Fish movement was calculated as x and y coordinates per frame at a frame rate of 40 fps. Median velocity and time active were determined for each fish for the entire 50-min trial. Time active was determined by the cumulative time a fish exhibited a swimming velocity above a threshold of 1.75 cm/s, divided by the total time of the video and expressed as a

percentage. As described in previous studies, this threshold is used to minimize noise and body wobbles (Tang et al., 2017; Tang and Fu 2019; Tang and Fu, 2020). Accuracy of tracking using EthoVision software was confirmed by watching at least three randomly selected tracks (i.e. post-analysis videos that showed the software determined location of the fish over the actual video itself) from each week.

2.5. Statistical analyses

All statistical analyses were performed in R (http:// www.R-project.org) using the RStudio interface (v 3.5.1). A predetermined α of 0.05 was used for all statistical tests. Two-way ANOVAs with sampling week and ploidy as fixed factors were used to determine the main effects of week and ploidy on all response variables. The assumptions of normality and homogeneity of variance of residuals were visually assessed using a Q-Q plot and a fitted vs. residuals plot, respectively. Weight and total length were log transformed, and hepatosomatic index and percentage time active were arcsine transformed prior to statistical analysis to account for normality and homogeneity of residuals. When significant effect of week was detected, a Tukey's post-hoc was used to determine differences between weeks. When the interaction between ploidy and week was significant, a Tukey's post hoc test was used to determine differences between ploidies for each week. For morphometric data (weight, length, SGR, FE, HSI, K and, deformities) tanks were used as sampling unit. For metabolic and activity data (RMR, MMR, FAS, median velocity, and percent time active), fish were randomly selected from growth trial tanks and individual fish was used as the sampling unit, since fish were placed in individual respirometers and buckets/ trash cans for metabolic and activity trials, respectively. All values are presented in the mean ± 1 standard deviation, unless otherwise stated.

3. Results

3.1. Survival, growth and feed efficiency

Overall, there were four total mortalities, three from 12 N tanks and one from 8 N tanks. Thus, the percent cumulative mortality was 0.67% and 2% for 8 N and 12 N sturgeon, respectively. There was at most, only one mortality per tank.

In general, triploid white sturgeon were smaller (lower weight and shorter) than their diploid counterparts starting at week 9 and this trend continued throughout the duration of the experiment. Body weights were significantly different between weeks ($F_{5,60} = 3385.7$, $p < 2.2 \times 10^{-16}$) and ploidies ($F_{1,60} = 42.7$, $p = 1.6 \times 10^{-8}$) Fig. 1. Moreover, there was a significant interaction between week and ploidy ($F_{5,60} = 6.0$, $p = 1.4 \times 10^{-4}$); body weights were similar during week 0 through 6, but triploid sturgeon weighed less than diploid sturgeon during weeks 9 through 15. On average, triploid white sturgeon weighed 13.1%, 14.9%, and 16.1% less than diploid sturgeon at week 9, 12, and 15, respectively. Additionally, total length was affected by both week ($F_{5,60} = 2946.1$, $p < 2.2 \times 10^{-16}$) and ploidy ($F_{1,60} = 45.9$, $p = 6.1 \times 10^{-9}$), with a significant interaction between week and ploidy ($F_{5,60} = 3.8$, $p = 0.005$) Figure 1. Like body weights, total length of triploid sturgeon was lower than diploid sturgeon at weeks 9 through 15.

Both specific growth rate (SGR) and feed efficiency (FE) showed similar trends: a decrease over time and lower values in triploid sturgeon

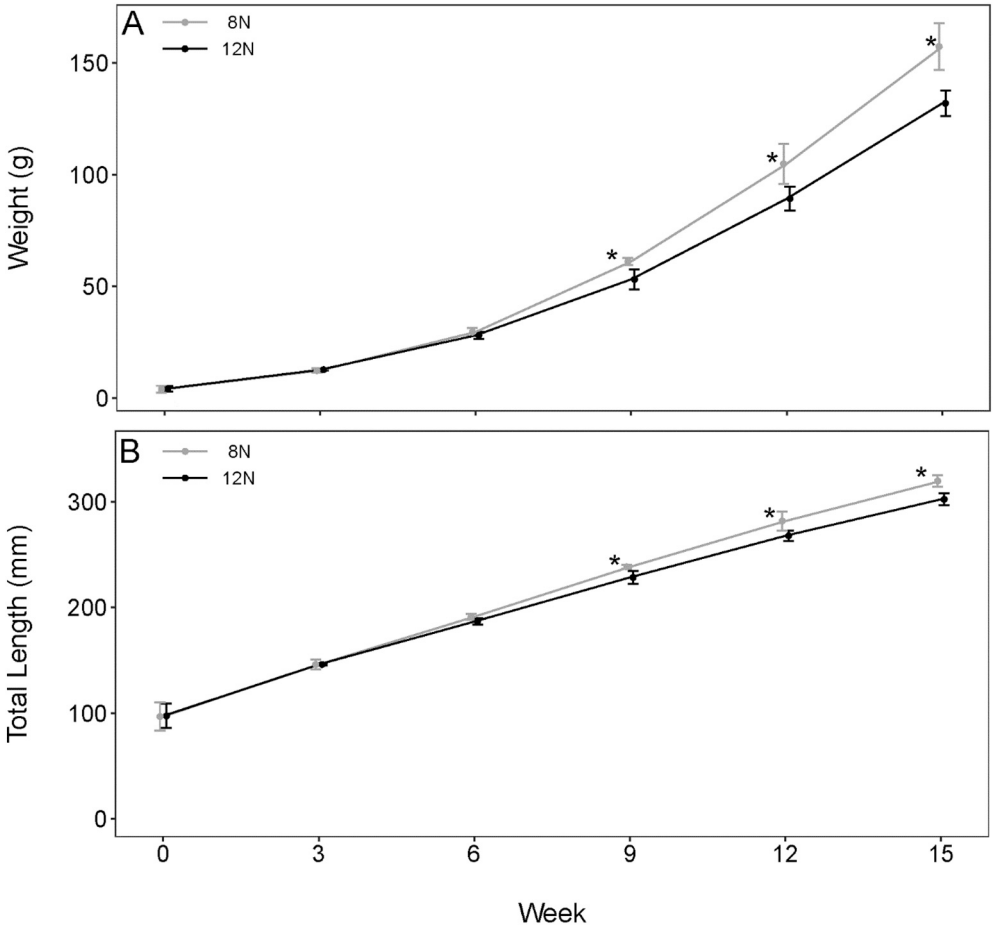

Fig. 1. Weight (A) and total length (B) of diploid (8 N) and triploid (12 N) white sturgeon across a 15-week growth trial. An asterisk (*) denotes a significant difference between ploidies within each week of sampling ($p < 0.05$). At week 0, sturgeon were two months post hatch. n = 100 fish/ploidy for week 1 and 7 tanks/ploidy for each subsequent week.

compared to diploid sturgeon. SGR decreased over time ($F_{4,60} = 226.5$, $p = 2.0 \times 10^{-16}$) and was significantly lower in triploid white sturgeon compared with diploid sturgeon ($F_{1,60} = 7.0$, $p = 0.01$) Fig. 2. FE also decreased with time ($F_{4,60} = 148.8$, $p < 2.2 \times 10^{-16}$) and was lower in triploid sturgeon ($F_{1,60} = 8.5$, $p = 0.005$), and there was a trend that ploidies differed in FE across week (week x ploidy interaction: $F_{4,60} = 2.1$, $p = 0.09$).

3.2. Aerobic scope

Overall, metabolic rate was similar between ploidies throughout the growth trial. Routine metabolic rate (RMR) was significantly affected by week ($F_{2,86} = 49.1$, $p = 5.9 \times 10^{-15}$) but not ploidy ($F_{1,86} = 0.3$, $p = 0.58$) Fig. 3. Maximum metabolic rate (MMR) also differed between weeks ($F_{2,86} = 94.2$, $p = 2.0 \times 10^{-16}$) but not between ploidies ($F_{1,86} = 0.05$, $p = 0.83$). Factorial aerobic scope was significantly different between weeks ($F_{2,86} = 3.9$, $p = 0.02$), and only during week 15 were ploidies significantly different, with triploid white sturgeon exhibiting a factorial aerobic scope that was, on average, 19% lower compared to diploid sturgeon ($F_{1,27} = 4.2$, $p = 0.05$). The mean absolute aerobic scope was 190.8 and 146.1 mg O_2/kg/h for 8 N and 12 N sturgeon, respectively, during week 15.

3.3. Fish condition and deformities

The potential for energy storage appears similar between diploid and triploid white sturgeon, as hepatosomatic index (HSI) and condition factor (K) did not differ between ploidies Fig. 4. HSI was significantly different across weeks ($F_{2,24} = 13.3$, $p = 1.3 \times 10^{-4}$) but did not differ between ploidies ($F_{1,24} = 0.1$, $p = 0.81$), and there was no significant interaction between week and ploidy ($F_{2,24} = 0.5$, $p = 0.62$). Condition factor also changed across weeks ($F_{5,60} = 64.2$, $p < 2.2 \times 10^{-16}$), but was not affected by ploidy ($F_{1,60} = 0.8$, $p = 0.37$), and there was no significant interaction between week and ploidy ($F_{5,60} = 1.4$, $p = 0.24$). Both HSI and K increased over time.

Triploid white sturgeon demonstrated an impaired development compared to diploid sturgeon, as evidenced by the number of deformities present Fig. 5. Throughout the duration of the growth trial, triploid sturgeon exhibited a higher percentage of deformities ($F_{1,60} = 6.9$, $p = 0.01$) and the percentage of deformities increased over time ($F_{5,60} = 3.9$, $p = 0.003$). Over the duration of the trial, the mean deformity rate was 6.4% and 9.5% for 8 N and 12 N sturgeon, respectively. The types of deformities observed throughout the experiments were missing pectoral fins, underdeveloped pectoral fins, and scoliosis.

3.4. Swimming activity

The swimming activity of white sturgeon was similar between ploidies, except during week 1 Fig. 6. Over the 50-min video, both ploidies spent about the same percentage of time active ($F_{1,87} = 1.7$, $p = 0.20$); additionally, percentage time active did not significantly change over the course of the 15-week trial ($F_{2,87} = 3.0$, $p = 0.06$). In general, median swimming velocity of the 50-min video also decreased over the 15-week trial ($F_{2,87} = 14.1$, $p = 4.8 \times 10^{-6}$) but did not differ between ploidies ($F_{1,87} = 0.2$, $p = 0.69$), with no significant interaction between week and ploidy ($F_{2,87} = 2.6$, $p = 0.08$).

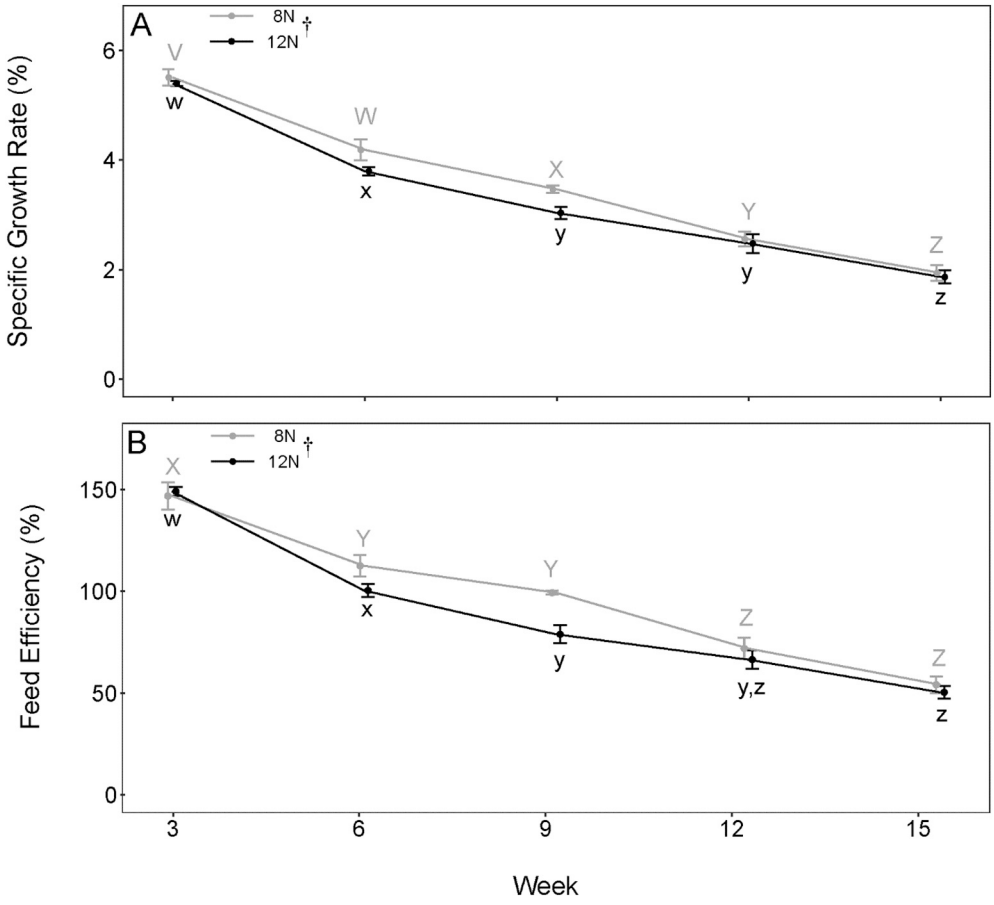

Fig. 2. Specific growth rate (A) and feed efficiency (B) of diploid (8 N) and triploid (12 N) white sturgeon across a 15-week growth trial. A dagger (†) denotes a significant main effect of ploidy ($p < 0.05$). Different letters denote significant differences across weeks for 8 N tanks (grey upper-case letters) and 12 N tanks (black lower-case letters) ($p < 0.05$). At week 0, sturgeon were two months post hatch. n = 7 tanks/ploidy for each week.

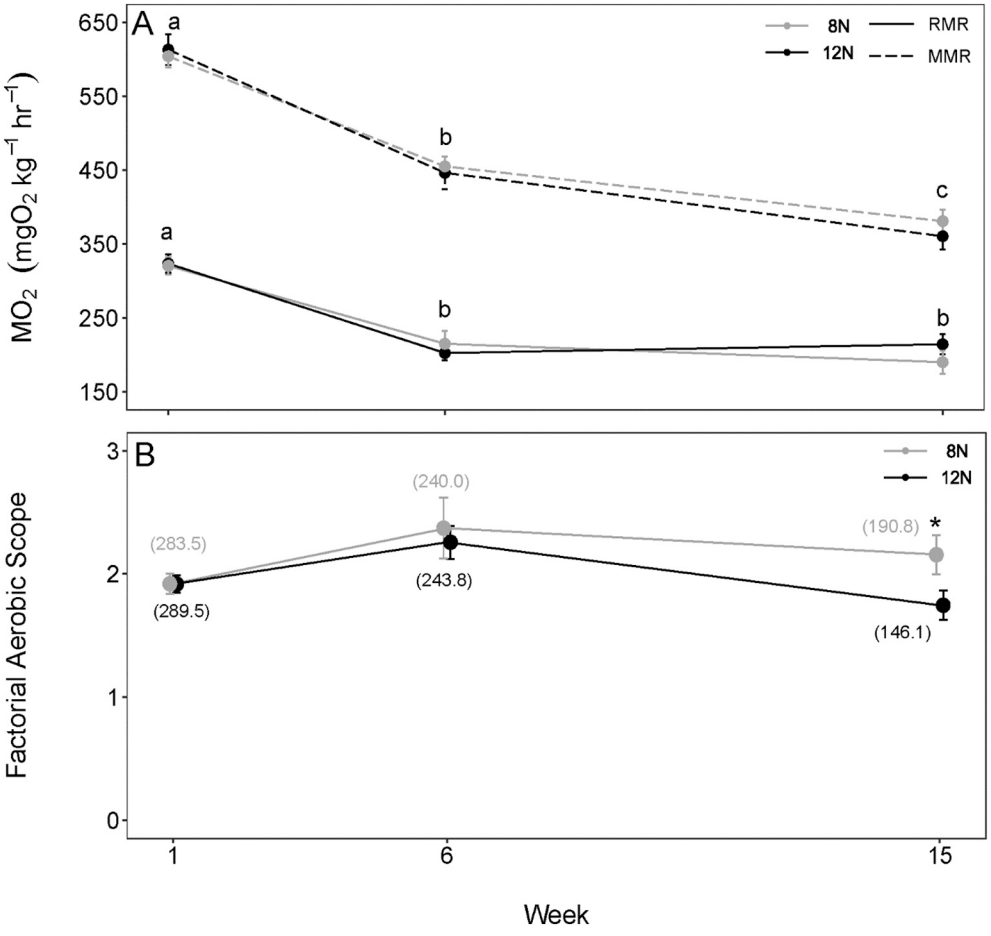

Fig. 3. Mass-specific metabolic rate (MO_2; A) and factorial aerobic scope (B) of diploid (8 N) and triploid (12 N) white sturgeon. Multiple measurements were taken over 22 h for baseline (RMR, routine metabolic rate) prior to manual chasing. Metabolic rate measurements were then taken immediately (MMR, maximum metabolic rate) following a chase protocol. An asterisk (*) denotes a significant difference between ploidies within each week of sampling ($p < 0.05$). Different letters denote significant differences across weeks for RMR and MMR separately ($p < 0.05$). Numbers in parantheses represent the mean absolute aerobic scope for 8 N (grey) and 12 N sturgeon (black) for each time point in mg O^2 kg^{-1} h^{-1}. At week 0, sturgeon were two months post hatch. n = 15–16 fish per ploidy for weeks 1 and 6, n = 14–15 fish per ploidy for week 15.

4. Discussion

Since previous studies indicated a lower aerobic capacity in triploid white sturgeon, we investigated the effect of triploidy on white sturgeon growth, condition, deformities, and spontaneous swimming activity, and aerobic scope to determine how energy allocation to these key biological processes might be impacted by polyploidy. In the current study, aerobic scope was lower in triploid white sturgeon at week 15. By this point in the growth trial, triploid white sturgeon had lower weight and lengths compared to diploid sturgeon. This difference may be due to the lower aerobic scope, which would limit the energy available for processes beyond maintenance. Triploid white sturgeon may also have different feed requirements, which could explain differences in feed efficiency and deformities, when compared to diploid sturgeon. Triploid white sturgeon do not appear to mitigate these differences in aerobic energy metabolism by altering condition or swimming activity.

4.1. Growth of diploid and triploid white sturgeon

In general, triploidy appears to reduce growth in juvenile white sturgeon. Beginning at week 9 (about-4 months post hatch) and continuing through week 15 (about 5.5 months post-hatch) of the growth trial, both weight and length were lower in triploid white sturgeon than in diploid sturgeon, indicating a lower growth rate. While one of the primary motivations for utilizing triploid fishes in aquaculture is the potential for increased growth, there are contradictory reports about the effect of triploidy on growth, as it seems largely dependent on species (see Tiwary et al., 2004 and Maxime, 2008 for reviews). In white sturgeon specifically, previous studies provided initial evidence that triploid white sturgeon had lower growth rates than diploid sturgeon, especially when chronically exposed to elevated water temperature (Leal et al., 2019). Our results indicate that even under well-established optimal tank conditions (Van Eenennaam et al., 2004) and feeding regimens (Lee et al., 2014), triploid white sturgeon still underperform compared to their diploid counterparts by at least 4 months post-hatch (week 9 in current study).

The lower weights and lengths exhibited by triploid white sturgeon may be due to a reduced feed efficiency. Specific growth rate (SGR) and feed efficiency (FE) were lower in triploid white sturgeon than in diploid white sturgeon, and the greatest difference between the ploidies occurred at about 4 months post-hatch (week 9). Both ploidies demonstrated similar SGR and FE at weeks 12 and 15; yet this is likely due to triploid sturgeon being fed a higher rate based on a lower body weight. Higher feed rates are likely to result in higher feed efficiencies (Hung et al., 1989; Rad et al., 2003); however, despite being fed a higher rate after week 9, triploid white sturgeon were unable to attain weights similar to their diploid counter parts, and the disparity in weight between ploidies continued to increase over time.

4.2. Aerobic scope of diploid and triploid white sturgeon

Triploidy reduced aerobic scope in white sturgeon, but the effect of triploidy was not evident until later in development. In the current study, triploid white sturgeon did not demonstrate a lower aerobic scope until week 15 (about 5.5 months post-hatch). Previous work with triploid white sturgeon showed reduced aerobic scope compared to diploid sturgeon at 3 months post-hatch (Leal et al., 2020). The discrepancy in the time for the difference in aerobic scope to manifest in these two experiments may be linked to parental inheritance. Mass specific metabolic rate is influenced by parental genetics, especially during early

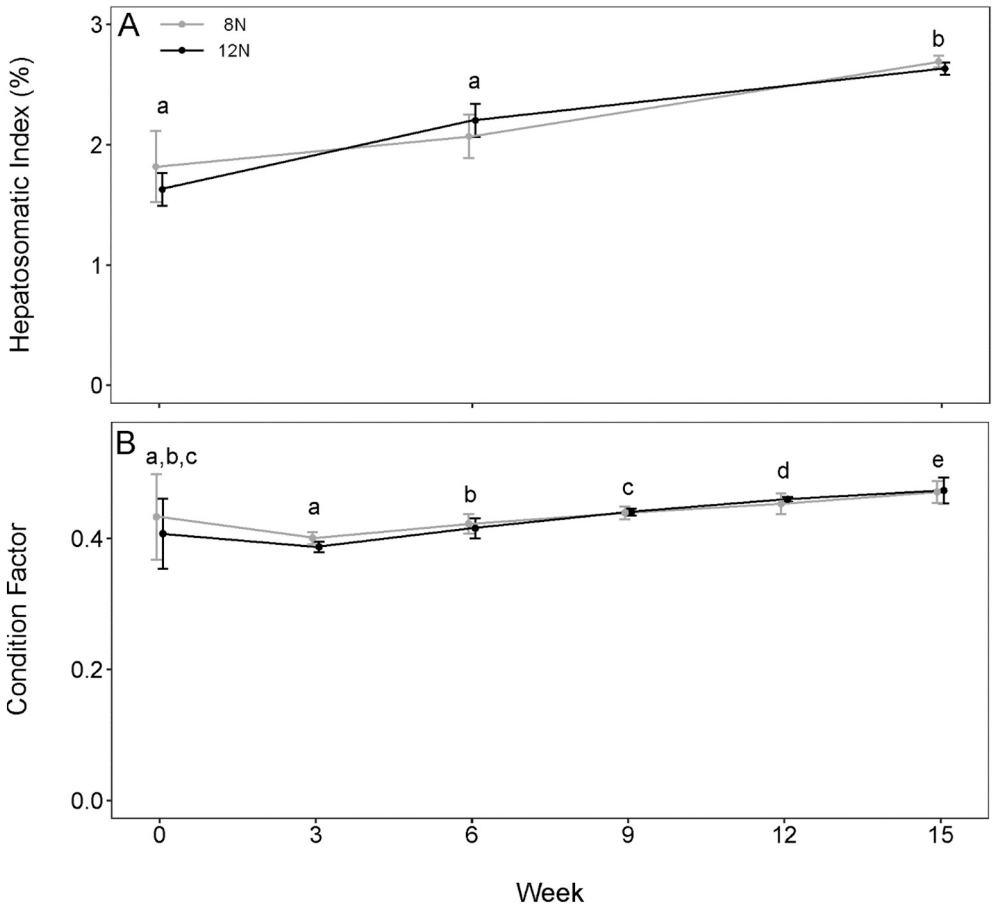

Fig. 4. Hepatosomatic index (A) and condition factor (B) of diploid (8 N) and triploid (12 N) white sturgeon across a 15-week growth trial. Different letters denote significant differences across weeks for both ploidies ($p < 0.05$). At week 0, sturgeon were two months post hatch. n = 7 tanks/ploidy for each week.

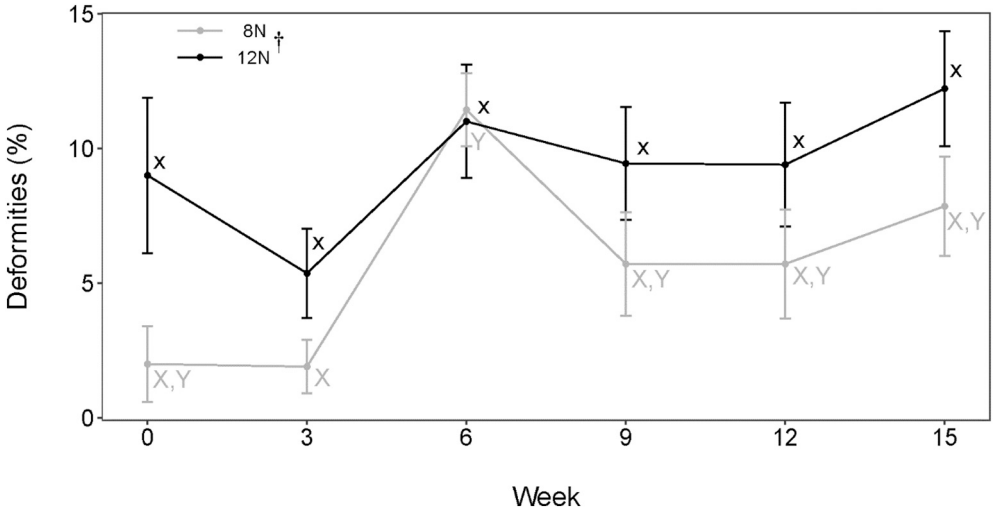

Fig. 5. Percentage of deformities of diploid (8 N) and triploid (12 N) white sturgeon across a 15-week growth trial. A dagger (†) denotes a significant main effect of ploidy ($p < 0.05$). Different letters denote significant differences across weeks for 8 N tanks (grey upper-case letters) and 12 N tanks (black lower-case letters) ($p < 0.05$). At week 0, sturgeon were two months post hatch. n = 7 tanks/ploidy for each week.

life stages (Pakkasmaa et al., 2005; Fossen et al., 2019). Also, the methods of triploid induction differed between the studies, with heat shock being used in the current study and a combination of ageing and vigorous stirring of eggs in a past study (Leal et al., 2020). Several studies have shown lower survival in heat shocked embryos during triploid induction (reviewed by Piferrer et al., 2009). An early die-off in the triploid sturgeon from heat shock could have resulted in only the "strongest" fish being used for experimentation, creating minimal differences between ploidies. Alternatively, the lack of a difference in aerobic scope between ploidies may refute the hypothesis that a reduction in SA:V reduced oxygen diffusion and, thus, aerobic metabolism. If SA:V were the sole underlying cause of all physiological

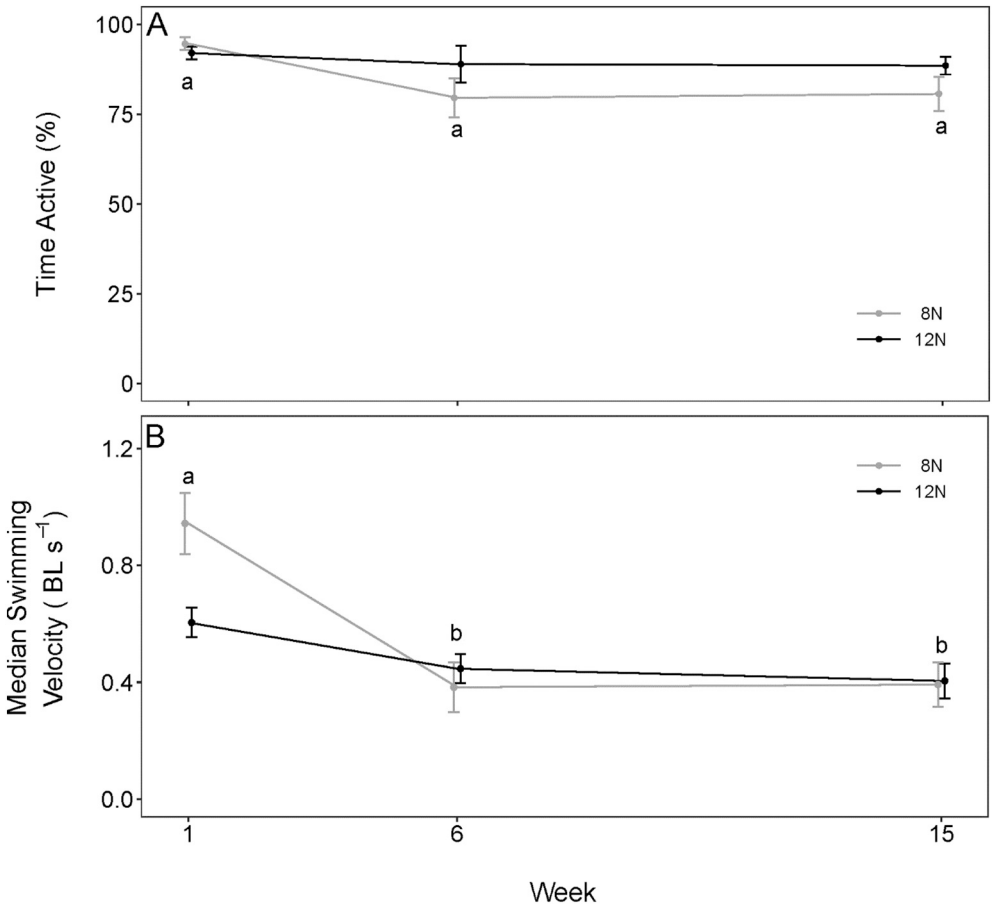

Fig. 6. Percent time active (A) and median swimming velocity (B) of diploid (8 N) and triploid (12 N) white sturgeon across a 15-week growth trial. Different letters denote significant differences across weeks for both ploidies ($p < 0.05$). At week 0, sturgeon were two months post hatch. n = 15–16 fish per ploidy and time point.

differences between diploid and triploid fishes, we would expect differences in aerobic metabolism across all life stages and species; however, that is not the case in the current study or when comparing species (Tiwary et al., 2004; Maxime, 2008). While SA:V may impact oxygen diffusion, there are likely other mechanisms underlying the differences in aerobic scope between ploidies. For example, triploid white sturgeon may have compensatory mechanisms for dealing with a reduced SA:V, such as number and localization of mitochondria. Additionally, gene dosage and compensation remain to be areas that should explored further to better understand physiological differences between diploid and triploid white sturgeon.

Nonetheless, these data along with evidence indicating that juvenile triploid white sturgeon have a lower maximum cellular aerobic capacity (Leal et al., 2019) indicate a lower aerobic capacity by about 5.5 months post-hatch and, potentially less available energy for processes beyond maintenance. Additionally, previous studies have found evidence of a lower aerobic capacity in triploid salmonids when compared with their diploid counterparts, such as a reduced factorial aerobic scope in triploid brown trout at elevated temperatures (Altimiras et al., 2002), a lower metabolic rate following exercise in triploid brook trout (Hyndman et al., 2003), and a higher estimated SMR in triploid brook charr (O'Donnell et al., 2017).

The difference in aerobic scope could provide another potential explanation for the difference in growth between diploid and triploid white sturgeon. In the current study, triploid white sturgeon exhibited a lower aerobic scope at week 15, but not week 1 or 6. It is unknown whether aerobic scope differed among ploidies at week 9 (when weights and lengths first differed) since whole animal metabolism was not measured at week 9, but the difference at week 15 might, in some part, account for the disparity in growth between diploid and triploid sturgeon. The 16.1% reduction in weight in triploid sturgeon compared to diploid sturgeon is similar to the 19% difference in aerobic scope at week 15. A similar difference of a 11–18% lower aerobic scope in triploid white sturgeon was found in a previous study and this study also measured a 14% lower erythrocyte surface area to volume ratio (Leal et al., 2020). It is possible that a reduction in SA:V may limit the rate of diffusion and impair diffusion-limited process, such as aerobic metabolism, which relies on oxygen diffusion. A reduced aerobic scope would leave a fish with less energy for processes besides maintenance, such as growth. However, because differences in aerobic scope were only demonstrated in week 15, more research is required to fully understand the physiological mechanism for the differences in growth and metabolism in diploid and triploid sturgeon and why comparisons of diploid and triploid physiology differ greatly between species.

4.3. *Condition and deformities of diploid and triploid white sturgeon*

Diploid and triploid white sturgeon did not differ in their hepatosomatic index or condition factor (K), suggesting there is likely no difference between ploidies in their energetic status or condition. Condition factor provides a quantitative evaluation of the robustness of an individual fish (Williams, 2000). Previous research has provided mixed results on the effect of triploidy on K. For example, triploid rainbow trout had higher K (Lincoln and Scott, 1984), triploid Chinese catfish had similar K (Qin et al., 1998), and triploid brook charr had lower K (Sacobie et al., 2016) when compared to their diploid counterparts. K values reported in the present study are comparable to those previously reported for juvenile white sturgeon (Hung and Lutes, 1987). Although

the absolute values of length and weight were lower in triploid sturgeon, the proportion between the two metrics (i.e. K) was similar to their diploid counterparts, suggesting triploid sturgeon exhibited a similar but delayed growth trajectory compared to diploid sturgeon. Hepatic somatic index (HSI) has been shown to be either equal (Kızak et al., 2013), or lower (Lincoln and Scott, 1984) in triploid fish compared to diploid fish. HSI values exhibited by sturgeon in this experiment match previously reported data for juvenile white sturgeon (Hung and Lutes, 1987). Additionally, previous studies have correlated HSI and K to energy status in multiple fish species including three-spined stickleback (*Gasterosteus aculeatus*), summer flounder (*Paralichthys dentatus*), striped bass (*Morone saxatilis*), and Atlantic croakers (*Micropogonias undulates*) (Chellappa et al., 1995; Schloesser and Fabrizio, 2017). Based on our results, there is indirect evidence that energy stores are likely comparable between diploid and triploid white sturgeon, but further research on the link between ploidy and energy status is required.

Despite having a similar K to diploid sturgeon, triploid white sturgeon exhibited an overall higher rate of deformities, which may be due to the process of triploidy induction or triploidy itself. A higher rate of deformities has also been reported in many triploid salmonid species (reviewed by Fraser et al., 2012). The triploid state itself may result in higher deformities. For example, lower jaw deformities were found exclusively in triploid Atlantic salmon (*Salmo salar*) (Sutterlin et al., 1987).; however, Sutterlin et al. (1987) also found similar rate of gill abnormalities in diploid and triploid Atlantic salmon that had been heat shocked, suggesting the process of heat shock results in deformities in general (triploidy in the current study was induced through heat shock). Other studies have attributed higher deformity rates in triploid fishes to differences in mineral requirements (Fjelldal et al., 2011; Peruzzi et al., 2018; Sambraus et al., 2020). While the underlying cause of higher rates of deformities remains unclear, it could be a disadvantage of utilizing triploid white sturgeon in some aquaculture operations. What is even more critical to study is if deformities continue into sub-adult and adult life stages.

4.4. Swimming activity of diploid and triploid white sturgeon

Triploidy appears to have a minimal effect on swimming activity in white sturgeon, providing evidence that diploids and triploids allocate a similar amount of energy to basal swimming activity. Diploid and triploid white sturgeon spent about the same percentage of time swimming and had a similar median swimming velocity. Previous work with brook trout (*Salvelinus fontinalis*) showed similar tail beat frequencies between diploid and triploid trout during forced swimming, suggesting triploid trout make a similar effort to maintain a similar swimming speed (Stillwell and Benfey, 1996). Based on our data, in general, diploid and triploid white sturgeon do not differ in spontaneous swimming activity, despite differences in aerobic scope, yet more research is needed to determine if energy allocation to swimming activity differs between ploidies.

5. Conclusions

Triploid fish and shellfish aquaculture is popular, especially when the resulting sterility results in increased growth rates. Yet, triploidy, in some species, appears to confer a physiological cost: reduced performance (i.e. lower growth and higher mortalities) at elevated temperatures. Triploid white sturgeon are fertile and can produce larger sized eggs for caviar (Van Eenennaam et al., unpubl data); however, there are also important potential costs of triploidy in sturgeon. While juvenile diploid and triploid white sturgeon are similar in many biological parameters (HSI, K, activity), triploid white sturgeon exhibited poorer growth performance and feed efficiency. It is a possibility that triploid white sturgeon have a higher feed requirement (which would explain the lower feed efficiency and growth); however, in the current study, even when fed a higher feed rate than their 8 N counterparts, 12 N sturgeon still had reduced growth rates compared to 8 N sturgeon. Higher growth rate is a potential benefit of triploidy for some species, such as Nile tilapia (Brämick et al., 1995), and is one of the main interests in producing sterile triploid fish. Yet it appears triploidy does not confer the benefit of higher growth rates in juvenile triploid white sturgeon. Due to the late maturation in captive white sturgeon females (age 7–10 years) whether this lower growth and feed efficiency in the juvenile stage carries over into the sub-adult, and adult life stages requires further study. The higher percentage of deformities in triploid white sturgeon also presents another possible disadvantage associated with triploidy, but the 3% increase at the juvenile stage may not actually be detrimental to a large-scale caviar farm because of continual grading of juveniles. It appears that triploid females have a delayed maturation (Schreier and Van Eenennaam, unpublished data); however, a much larger, long-term (10 year) study is required to clearly determine if triploid white sturgeon females mature at similar ages to diploids, and whether they have similar caviar yields but consistently larger eggs.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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