Comparative Biochemistry and Physiology, Part A 229 (2019) 10–17

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Triploidy in white sturgeon (*Acipenser transmontanus*): Effects of acute stress and warm acclimation on physiological performance

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A B S T R A C T

Previous studies have demonstrated reduced performance in triploid fish when reared under suboptimal conditions, which may be the result of a higher susceptibility to stressors when compared to diploids. The goal of this project was to investigate differences in the capacity of diploid (8 N) and triploid (12 N) white sturgeon, *Acipenser transmontanus*, to respond to both warm acclimation (6-weeks of acclimation to either 18 or 22 °C) and a subsequent acute stress (10-min low water stress). Following the 6-week acclimation, fish were sampled either before or following an acute low water stress. Bioindices of the primary and secondary stress response, hematology and cellular metabolic status were measured. We also sought to determine if time to peak cortisol levels were similar between diploid and triploid sturgeon after exposure to a severe acute stressor (netting stress). While both ploidies had similar primary and secondary responses to acute stress, both with and without warm acclimation, warm acclimation impacted the ability of diploid and triploid white sturgeon to mount a typical stress response to an acute stressor. In response to warm acclimation, triploids exhibited little change in branchial lactate dehydrogenase activity, while diploids increased activity. After exposure to an acute water reduction stress, diploids increased citrate synthase activity, yet triploids showed a decrease in activity. Differences in metabolic enzyme activity in response to warm acclimation and acute stress suggest triploid white sturgeon may have a reduced cellular metabolic capacity under chronic and acute stress, which may impact performance of triploid sturgeon in suboptimal conditions.

1. Introduction

Triploidy, or the possession of an additional set of chromosomes, has been induced in multiple species of cultured fishes, such as Atlantic salmon (*Salmo salar*), Coho salmon (*Oncorhynchus kisutch*), brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*) (reviewed by Benfey, 1999). Inducing triploidy typically results in a sterile fish. In aquaculture, the benefits of producing sterile fish include improved growth rates and prevention of escapees mating with wild individuals (Maxime, 2008). There is growing evidence that there are costs associated with triploidy as triploids often underperform in suboptimal conditions when compared to diploids (e.g. Ojolick et al., 1995; Hyndman et al., 2003; Sambraus et al., 2017). Although a reduced performance in triploids in suboptimal environments has been documented in salmonids, the biological mechanism causing this poorer performance is still unclear, requiring further investigation, and may be related to differences in cell size in triploid fishes (Benfey, 1999).

Triploids have 50% more genetic material than diploids and accommodate for this larger genome by increasing nuclear and cellular volume. This increase in cell size often occurs with a concomitant reduction in total cell numbers (Benfey, 1999). The difference in cell size and number in triploids results in an overall reduction in cellular surface area to volume ratios (SA:V), which likely impacts many physiological processes, such as diffusion and signal transport throughout an organism (Benfey, 1999). Hence, triploid physiology may be impaired by a reduced cellular SA:V, potentially accounting for the difference in performance observed between diploid and triploid salmonids under suboptimal conditions. As there is interest in increasing the use of triploids in aquaculture, the importance of attaining a better understanding of the physiology of triploids is apparent.

White sturgeon (*Acipenser transmontanus*) diploids contain eight copies of each of their chromosomes (8 N), and therefore produce gametes with four sets of chromosome copies. When the second polar body is retained during meiosis, a 12 N offspring is produced with an additional set of four chromosomes (referred to as a triploid, Gille et al., 2015). Triploid white sturgeon have been documented on white sturgeon farms, without practices to intentionally produce triploids (Schreier et al., 2013). Unlike triploid salmonids that are sterile, triploid white sturgeon are fertile and can produce viable eggs and offspring. When a triploid is crossed with a diploid, resultant offspring have a genome size intermediate of diploid and triploid (i.e. 10 N), making them a suitable model species to ascertain the effects of ploidy separately from the effects of sterility (Drauch Schreier et al., 2011).

To date, very little is known about the physiology of triploid sturgeon. While many studies have compared physiological responses to various stressors in diploid and triploid salmonids (reviewed by Benfey,

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https://doi.org/10.1016/j.cbpa.2018.11.006
Received 15 October 2018; Accepted 8 November 2018
Available online 14 November 2018
1095-6433/

1999; Maxime, 2008), physiological comparisons between sturgeon of different ploidies has received little attention, with only two published papers (Beyea et al., 2005; Leal et al., 2018), both of which have demonstrated some differences between physiological responses to stressors. Triploid shortnose sturgeon (*Acipenser brevirostrum*) have been shown to have a higher secondary stress response compared to diploids (Beyea et al., 2005). Previous experiments with 8 N (diploid) and 10 N (intermediate ploidy) white sturgeon have shown similar generalized stress responses to warm acclimation but differences in metabolic enzyme activities both prior to and after warm acclimation (Leal et al., 2018). Furthermore, current research concerning diploid and triploid fishes has failed to address physiological responses to multiple stressors across multiple time scales (i.e. chronic vs. acute stress, independent and simultaneous).

Previous work in our lab demonstrated that metabolic enzyme activities involved in both aerobic (citrate synthase) and anaerobic (lactate dehydrogenase) pathways of 8 N and 10 N white sturgeon differed, especially after acclimation to a higher temperature, providing evidence that 10 N sturgeon may have a reduced cellular aerobic capacity (Leal et al., 2018). Given this potential difference in 8 N and 10 N cellular energy metabolism and the paucity of information on sturgeon triploid physiology, we aimed to assess the primary and secondary stress responses and cellular metabolic alterations of an acute stressor following six weeks of acclimation to elevated temperatures in diploid (8 N) and triploid (12 N) white sturgeon. We also examined the primary stress response and timing to peak cortisol levels of diploid and triploid white sturgeon after being subjected to a severe acute stressor. Since mounting a physiological stress response is an energetically demanding process, we hypothesized that triploid white sturgeon would have less energy to cope with stress, due to a reduction in cellular SA:V in triploids limiting oxygen diffusion and, therefore cellular metabolism. We predicted that triploids (12 N) would be more susceptible to an acute stressor marked by elevated biomarkers of physiological stress, especially after exposure to chronically elevated temperatures. We also predicted that triploid sturgeon would take longer to mount a peak cortisol response to an acute stressor.

2. Materials and methods

We conducted two experiments to study 1) the stress and metabolic response of diploid and triploid white sturgeon to an acute stress (water reduction) after warm acclimation (+4 °C) (Experiment I) and 2) the primary stress response and timing of cortisol release of diploid and triploid white sturgeon to a more severe acute stressor (netting) (Experiment II).

2.1. Fish source and husbandry

During both spring 2016 and 2017, white sturgeon ovulated eggs from one 8 N female and milt from one 8 N male were acquired from domesticated broodstock at a northern California sturgeon farm each year. Broodstock were reared and spawned under typical culture conditions as described in Van Eenennaam et al. (2004). Ploidy of the broodstock was verified using flow cytometry as described in Schreier et al., (2013). Eggs and milt underwent standard fertilization and de-adhesion procedures (Van Eenennaam et al., 2004) with slight alterations. Two methods were used to produce triploid (12 N) sturgeon: aging ovulated eggs in coelomic fluid for 6 h prior to fertilization (Experiment I; 2016) or by vigorously mixing newly fertilized eggs with a feather to generate a physical shock (Experiment II; 2017). Triploids naturally occur on farms without induction practices in place (Schreier et al., 2013), and both induction methods used in our experiments are mild compared to traditional triploid induction methods, such as heat and pressure shock.

Fertilized eggs were kept 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, CA, USA. Eggs were incubated in McDonald jars, in a flow-through hatchery system (15.5 ± 0.5 °C). After hatch, larvae were transported to Center for Aquatic Biology and Aquaculture (CABA) facilities at the University of California, Davis. After yolk-sac depletion, fish experienced a typical feeding regime from larval stage through the juvenile stage as described in Leal et al. (2018). At 90–120 days post-hatch, ploidy of individual fish was verified using a Coulter counter and 8 N and 12 N fish were reared in separate tanks with flow-through well water (ambient temperature 18.5 ± 0.5 °C) until experimentation as juvenile fish (age 12 months for Experiment I and age 10 months for Experiment II). Experiments I and II each used a different year class of sturgeon, but the source and husbandry conditions were the same for each year class until the time of experimentation. All husbandry, handling, and sampling procedures followed the protocol approved by the UC Davis Institutional Animal Care and Use Committee (protocol #18329).

2.2. Experiment I design

Prior to the start of the 6-week temperature acclimation, 40 white sturgeon juveniles from each ploidy were tagged using a passive integrated transponder (PIT) tag as well as an external Floy tag (Floy Tag Inc., Seattle, USA). Diploid and triploid sturgeon were assigned different Floy tag colors, which allowed for easy ploidy identification during sampling. Internal PIT tags allowed for identification of individual fish and served as a secondary means of identification if a Floy tag was not retained. During tagging, each sturgeon was anesthetized using sodium bicarbonate buffered tricaine methanesulfonate (MS-222, 100 mg/l). PIT tags were injected into the left-side musculature near the dorsal fin, while Floy tags were injected in a similar location on the right-side musculature. During the tagging procedure, initial fork length and body weight were recorded (Table 1). Sturgeon were then placed in one of two tanks, separated by ploidy, and allowed to recover for one week.

After the recovery period, ten sturgeon from each ploidy were randomly assigned to one of two temperature acclimation treatments (2 replicate tanks/treatment), with a total of 20 sturgeon in each tank. Due to limitations on temperature control, only two replicate tanks per temperature were available for use in this experiment. Water temperature was recorded every hour by HOBO® temperature loggers

Table 1
Morphometric parameters of diploid and triploid white sturgeon prior to and after a 6-week acclimation in Experiment I (n = 20).

	Diploid		Triploid	
	18 °C-Acclimated	22 °C-Acclimated	18 °C-Acclimated	22 °C-Acclimated
Initial Weight (g)	974 ± 219	1017 ± 243	888 ± 248	953 ± 249
Initial Fork Length (cm)	47.3 ± 3.6	48.1 ± 3.9	46.5 ± 3.8	47.0 ± 3.9
Final Weight (g)[a]	1095 ± 260	1068 ± 271	872 ± 252	864 ± 250
Final Fork Length (cm)[a]	50.4 ± 3.5	50.7 ± 4.2	48.8 ± 3.7	48.8 ± 4.1
Specific Growth Rate (%/d)[a,b]	0.28 ± 0.34	0.11 ± 0.24	−0.04 ± 0.25	−0.24 ± 0.32

[a] denotes a significant difference between ploidies ($p < 0.05$).
[b] denotes a significant difference between acclimation temperatures ($p < 0.05$).

(Onset Computer Corporation, Bourne, USA) throughout the duration of the experiment. Control temperature acclimation tanks were maintained at a temperature of 18.4 ± 0.1 °C and warm temperature acclimation tanks were maintained at a temperature of 22.3 ± 0.3 °C. All tanks (18 and 22 °C-acclimated) were fed at the same rate based on the elevated temperature to ensure feed was not a limiting factor for growth in fish acclimated to 22 °C. During the acclimation period, sturgeon were fed a basic trout pelleted diet (Skretting, Tooele, USA; 40% protein, 12% fat) at 0.74% of body weight using 24-h automatic belt feeders. A feed rate of 0.74% body weight was chosen based on fish size and water temperatures (22 °C) from previously established aquaculture guidelines for white sturgeon (Van Eenennaam et al., 2004). Sturgeon were held in fiberglass tanks (122 cm diameter, 42 cm height, 490 l) supplied with degassed well-water at a flow rate of 0.61 ± 0.02 l/s. Sturgeon were allowed six weeks to acclimate to their respective tanks and temperatures prior to sampling.

Following the 6-week acclimation, sturgeon were sampled on two separate days; one 18 °C and one 22 °C-acclimated tank were sampled in the early morning on each day to control for diurnal cortisol changes. For each tank, five diploid and five triploid fish were netted and immediately placed in a lethal dose of buffered MS-222 (1000 mg/l). The remaining ten fish (five of each ploidy) were exposed to an acute stressor. Acute stress in white sturgeon was simulated using a 10-min water reduction stress, shown to elicit a typical neuroendocrine stress response in sturgeon (Lankford et al., 2005). The water was quickly lowered to expose the dorsal fins of the sturgeon. The water was held at this level for 10 min then fish were allowed a 30-min recovery period before sampling during which the water level was quickly raised back to the level it had been previously during the acclimation period. Blood and tissue sampling procedures followed Leal et al. (2018). Plasma and gill samples were frozen on dry ice and stored at −80 °C until analyses.

2.3. Experiment II design

Over the course of Experiment II, sturgeon were fed a basic trout pelleted diet at 1% of body weight (Skretting; 40% protein, 12% fat) using 24-h automatic belt feeders, but were fasted 24 h prior to experimentation. As before, feed rate was determined based on fish size and water temperature from established aquaculture feeding guidelines for white sturgeon (Van Eenennaam et al., 2004). Sturgeon were held in fiberglass tanks (37 cm diameter, 11 cm height, 11.8 l), which were supplied with degassed well-water at a flow rate of 0.207 ± 0.002 l/s (mean temperature ± S.D.: 18.3 ± 0.1 °C).

A total of eight tanks were used on four separate sampling days to increase sample size (i.e. tanks were replicated over days). Six sturgeon were transferred to each tank according to ploidy and allowed to recover for a minimum of 52 h. Preliminary trials conducted in our laboratory demonstrated that a 48-h recovery time was sufficient for both ploidies to return to baseline cortisol levels after transfer to a new tank (data not shown). In order to elicit a severe stress response and to assess the time to peak cortisol levels in both ploidies, an acute netting stress experiment was conducted using the 2017 year class of sturgeon. In this experiment, sturgeon were exposed to a five-minute netting stressor (Lankford et al., 2005). A netted screen was held stationary in the tank while a net was used to circle around the tank to catch all the sturgeon in the tank. The net containing all sturgeon was then held underwater for five minutes against the screen to prevent fish from escaping. Sturgeon were then released and allowed to recover for 15, 30, or 60 min and then subsequently sacrificed and sampled. Baseline (Time 0) samples were also taken prior to the acute stress. For each sampling day, tanks were randomly assigned to ploidy and sampling time post-stress such that fish were only sampled at one time point per tank to minimize the effect of handling on the stress response. Blood and tissue sampling procedures were the same as Experiment I described above. On each sampling day, Time 0 tanks were sampled first.

2.4. Hematological and metabolic enzyme assays

The primary stress response of white sturgeon was assessed by measuring plasma cortisol levels according to Leal et al. (2018). Briefly, cortisol was measured using commercially available ELISA kits (Neogen Corporation, Lansing, USA). Bioindicators of the secondary stress response, including total erythrocyte counts (RBC), hematocrit (Hct), hemoglobin (Hb), mean erythrocyte volume (MEV), mean erythrocytic hemoglobin concentration (MEH), mean erythrocytic hemoglobin content (MEHC), and plasma glucose and lactate, were determined following standard procedures (Leal et al., 2018). Plasma osmolality was measured using a vapor pressure osmometer (Westcor, Logan, USA).

Lactate dehydrogenase (LDH) is a metabolic enzyme that converts pyruvate and lactate and is often used as an indicator of anaerobic metabolism; while citrate synthase (CS) is enzyme involved in the citric acid cycle and is a bioindicator of cellular aerobic metabolism (Chandel, 2015). LDH and CS enzyme assays were measured as previously described by Leal et al. (2018), optimized for white sturgeon gill tissue using the same homogenization buffer. Briefly, gill tissue was homogenized in ice cold potassium phosphate buffer (50 mM, pH 7.5). The activity of LDH was determined by monitoring the conversion of NADH to NAD^+ spectrophotometrically (Biotek Synergy HT, Winooski, USA) at 340 nm in imidazole buffer (52.5 mM, pH 7.5) with 2.64 mM pyruvate. The activity of CS was determined by monitoring the production of DNTB [5,50-dithiobis (2-nitrobenzoic acid)] spectrophotometrically (Biotek Synergy HT) at 412 nm in imidazole buffer (50 mM, pH 8.2) with 1 mM oxaloacetate. All homogenates were run at two temperatures, both 18 °C and 22 °C, to match experimental treatments. Total protein of gill tissue was determined using a kit, optimized for white sturgeon gill tissue, with bovine serum albumin as the standard (Thermo Fisher Scientific, Waltham, USA). Activity is expressed as μmol of product produced per minute per g of fresh gill weight.

2.5. Statistical analysis

Data were analyzed in R (http://www.R-project.org) using the RStudio interface (v 3.3.0). For all statistical tests, an α of 0.05 was used as the significance level. Statistical relationships for experimental data for Experiment I were analyzed using a three-way analysis of variance (ANOVA) with acclimation temperature, stress (pre- and post-stress), and ploidy as fixed factors. For statistical analyses, individual fish served as the experimental unit but were nested within tank to account for any tank effects. Weight was included as a covariate when there was a clear trend between weight and the response variable and a three-way analysis of covariance (ANCOVA) was used. For Experiment II, replicate tanks (across multiple sampling days) served as the experimental unit for a two-way ANOVA with ploidy and time (baseline/time 0, 15, 30, 60 min post stress) as fixed factors.

Prior to running an ANOVA or ANCOVA, the assumptions of normality and homogeneity of variance of residuals were visually inspected using a Q-Q plot and a fitted vs. residuals plot, respectively. When residuals were not normally distributed, a log transformation was applied to the response variable. If variances were not homogenous between treatment levels, a generalized least squares model with a "varIdent" variance structure based on the treatment groups was used ("nlme" package; Pinheiro et al., 2016). All values presented are mean ± 1 s.d. unless otherwise stated. Significant differences between treatment levels were determined using a Tukey's post hoc test ("lsmeans" package; Lenth, 2016).

3. Results

3.1. Experiment I

Sturgeon weight ($F_{1,78} = 1.97$, $p = .16$) and fork length ($F_{1,78} = 1.33$, $p = .25$) did not differ between ploidies before the acclimation period

(Table 1). After the six-week acclimation period, triploid white sturgeon had significantly lower weights ($F_{1,76} = 15.29$, $p < .001$) and fork lengths ($F_{1,76} = 4.18$, $p = .04$) compared to diploids in both 18 °C- and 22 °C-acclimated fish (Table 1). Temperature acclimation ($F_{1,76} = 7.74$, $p = .007$) and ploidy ($F_{1,76} = 23.53$, $p < .001$) impacted specific growth rates, with 22 °C-acclimated triploid sturgeon demonstrating the lowest specific growth rates (Table 1). There was no significant interaction between ploidy and acclimation temperature for specific growth rate ($F_{1,76} = 0.07$, $p = .79$). Triploid sturgeon in both temperature acclimation groups demonstrated negative specific growth rates and reduced mean weights after the acclimation period (Table 1).

Plasma cortisol concentrations were measured as an indicator of the primary stress response. Exposure to an acute water reduction stressor resulted in significantly increased plasma cortisol concentrations ($F_{1,64} = 23.78$, $p < .001$, Fig. 1), with no differences between ploidies ($F_{1,64} = 2.05$, $p = .16$, Fig. 1). Acclimation temperature significantly affected plasma cortisol concentrations with higher cortisol concentrations in 22 °C-acclimated sturgeon ($F_{1,64} = 4.89$, $p = .03$, Fig. 1) for both pre-stress and post-stress sturgeon (i.e. no significant acute stress by acclimation temperature interaction: $F_{1,64} = 0.11$, $p = .74$).

Several hematological parameters differed between ploidies. Compared to diploid white sturgeon, triploid white sturgeon had lower total erythrocyte counts ($F_{1,64} = 90.06$, $p < .001$), total leukocyte counts ($F_{1,64} = 79.80$, $p < .001$), hematocrit ($F_{1,64} = 73.84$, $p < .001$), and hemoglobin concentrations ($F_{1,64} = 20.57$, $p < .001$). In contrast, triploid sturgeon demonstrated higher mean erythrocyte volume ($F_{1,64} = 75.00$, $p < .001$) and mean erythrocyte hemoglobin ($F_{1,64} = 73.94$, $p < .001$) compared to diploid sturgeon (Table 2). Both ploidies demonstrated similar trends in terms of the secondary stress response. Plasma glucose ($F_{1,64} = 4.63$, $p = .04$), lactate ($F_{1,64} = 10.06$, $p = .002$) and osmolality levels ($F_{1,64} = 6.41$, $p = .01$) were elevated after exposure to an acute water reduction stress; however, ploidy did not significantly affect plasma glucose ($F_{1,64} = 0.02$, $p = .88$), lactate ($F_{1,64} = 0.56$, $p = .46$) or osmolality ($F_{1,64} = 0.23$, $p = .64$; Table 2). Additionally, these secondary stress response bioindicators demonstrated no significant acute stress by ploidy (lactate: $F_{1,64} = 0.005$, $p = .95$; glucose: $F_{1,64} = 0.008$, $p = .93$; osmolality: $F_{1,64} = 0.12$, $p = .73$) or acute stress by acclimation temperature interactions (lactate: $F_{1,64} = 0.21$, $p = .65$; glucose: $F_{1,64} = 0.20$, $p = .65$; osmolality: $F_{1,64} = 0.05$, $p = .82$). Acclimation to 22 °C resulted in significantly elevated plasma lactate levels in both ploidies ($F_{1,64} = 15.96$, $p < .001$, Table 2) but not for glucose ($F_{1,64} = 0.05$, $p = .83$) or osmolality ($F_{1,64} = 1.08$, $p = .30$).

In both diploid and triploid white sturgeon, multiple bioindices of the secondary stress response tended to increase after exposure to an acute water reduction stressor in 18 °C-acclimated sturgeon; however, warm acclimation to 22 °C showed a different response for similar bioindices (Table 2). Specifically, there was a significant interaction between acute stress and acclimation temperature on hematocrit ($F_{1,64} = 12.32$, $p < .001$) and hemoglobin ($F_{1,64} = 7.55$, $p = .008$). Both hematocrit and hemoglobin increased after exposure to an acute stressor in sturgeon acclimated to 18 °C but decreased after exposure to an acute stressor in sturgeon acclimated to 22 °C. Acute stress affected total protein concentration per gram of gill tissue but this effect differed depending on acclimation temperature (significant acute stress by acclimation temperature interaction; $F_{1,63} = 9.62$, $p = .003$). For both diploid and triploid sturgeon, exposure to an acute water reduction stress in the 18 °C-acclimated sturgeon resulted in increases in total gill protein levels; however, after acclimation to 22 °C, exposure to an acute stressor did not alter total gill protein concentrations (Table 2). Mean erythrocyte hemoglobin (MEH) was the only hematological parameter in which diploid and triploid sturgeon responded differently to temperature acclimation (significant ploidy by temperature interaction, $F_{1,64} = 5.73$, $p = .02$). Diploid sturgeon showed no change in MEH after 22 °C acclimation, while triploid sturgeon demonstrated an increase in MEH (Table 2).

Acclimation temperature affected enzyme specific activities of citrate synthase (CS) and lactate dehydrogenase (LDH) in gill tissue of both ploidies ($F_{1,127} = 24.84$, $p < .001$ and $F_{1,128} = 4.68$, $p = .003$, respectively); however, the effect of acclimation temperature on LDH activity was different depending on ploidy (i.e. significant ploidy by acclimation temperature interaction: $F_{1,128} = 5.50$, $p = .02$). In both pre- and post-stress white sturgeon, triploid sturgeon showed very little change in LDH activity with 22 °C acclimation, while diploid sturgeon demonstrated an increase in LDH activity with 22 °C acclimation (Fig. 2). There was no effect of an acute water reduction stressor on LDH activity in either ploidy ($F_{1,128} = 1.89$, $p = .17$). The activity of CS in white sturgeon decreased with 22 °C acclimation consistently between diploids and triploids (no significant ploidy by acclimation temperature interaction: $F_{1,127} = 0.03$, $p = .56$). CS activity was also affected by exposure to an acute water reduction stressor and this effect differed between diploid and triploid sturgeon (significant ploidy by stress effect: $F_{1,127} = 4.12$, $p = .04$). Diploid white sturgeon exhibited an increase in CS activity, while triploid sturgeon demonstrated a decrease in CS activity after exposure to the acute stressor (Fig. 3).

3.2. Experiment II

The only morphometric parameter that differed between diploid and triploid white sturgeon in Experiment II was weight. Diploid

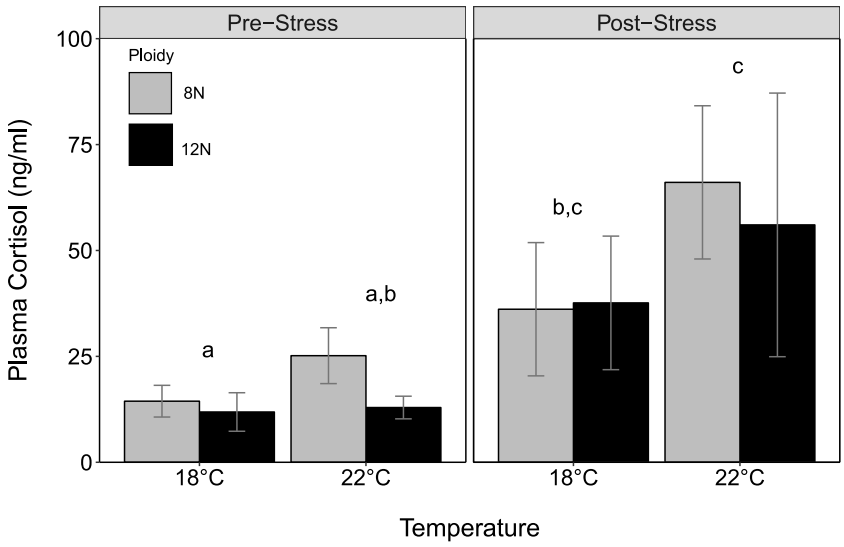

Fig. 1. Plasma cortisol concentrations (ng/ml) of 8 N (grey) and 12 N (black) white sturgeon before and 30 min after a 10-min water reduction stressor following a 6-week temperature acclimation to either 18 °C or 22 °C ($n = 10$ for each group). Different letters denote significant differences between acclimation treatments ($p < 0.05$).

Table 2
Bioindicators of the secondary stress response of diploid and triploid white sturgeon before and 30 min after a 10-min water reduction stress following a 6-week temperature acclimation ($n = 10$).

	Diploid				Triploid			
	Pre-Stress		Post-Stress		Pre-Stress		Post-Stress	
	18 °C-Acclimated	22 °C-Acclimated	18 °C-Acclimated	22 °C-Acclimated	18 °C-Acclimated	22 °C-Acclimated	18 °C-Acclimated	22 °C-Acclimated
RBC (10^6 µl^{-1}) †	0.72 ± 0.30	0.73 ± 0.12	0.79 ± 0.12	0.75 ± 0.13	0.42 ± 0.10	0.43 ± 0.07	0.52 ± 0.17	0.42 ± 0.06
WBC (10^4 µl^{-1})†	6.0 ± 2.3	5.9 ± 1.8	6.2 ± 1.3	6.2 ± 1.1	3.7 ± 0.7	3.5 ± 0.7	4.1 ± 1.0	4.0 ± 0.8
Hct (%)†, ǂ	26 ± $2^{a,b,c}$	29 ± 3^{a}	28 ± 3^{a}	27 ± $2^{a,b}$	22 ± 1^{d}	24 ± $2^{b,c,d}$	24 ± $2^{b,c,.d}$	23.5 ± $1^{c,d}$
Hb (g/dl)†, ǂ	6.6 ± $1.3^{a,b}$	6.9 ± $1.0^{a,b}$	7.7 ± 1.5^{a}	6.5 ± $0.5^{a,b}$	5.5 ± 1.5^{b}	6.3 ± $1.4^{a,b}$	5.9 ± 0.9^{b}	5.7 ± 0.8^{b}
MEV (fl/cell)†	401 ± 107	403 ± 64	364 ± 68	363 ± 51	554 ± 121	583 ± 67	496 ± 113	568 ± 70
MEH (pg/cell)*, †, •	97 ± $22^{a,b}$	96 ± $19^{a,b}$	97 ± $15^{a,b}$	88 ± 14^{a}	135 ± $27^{c,d}$	154 ± 26^{d}	120 ± $27^{c,d}$	138 ± $1^{c,d}$
MEHC (g/dl)	24.9 ± 4.0	23.8 ± 1.6	27.1 ± 5.3	24.3 ± 1.0	25.0 ± 6.3	26.6 ± 4.9	24.2 ± 2.3	24.3 ± 2.5
Plasma Glucose (mM)*	3.8 ± 0.3	3.7 ± 0.3	4.1 ± 0.7	4.2 ± 1.0	3.8 ± 0.3	3.7 ± 0.5	4.0 ± 1.1	4.3 ± 1.7
Plasma Lactate (mM)*, +	1.5 ± 0.5	1.9 ± 0.6	1.8 ± 0.5	3.4 ± 2.1	1.4 ± 0.4	1.9 ± 0.4	2.2 ± 2.3	3.0 ± 2.1
Plasma Osmolality (mOsm/kg)*	272 ± 3	276 ± 9	279 ± 4	281 ± 13	271 ± 4	275 ± 6	277 ± 9	278 ± 12
Total Gill Protein (mg/ml)†	3.4 ± 0.8^{a}	4.1 ± 0.6^{b}	4.1 ± 0.5^{b}	3.9 ± $0.5^{a,b}$	3.3 ± 0.6^{a}	3.6 ± 0.04^{b}	3.9 ± 0.05^{b}	3.6 ± $0.6^{a,b}$

A dagger (†) denotes a significant difference between ploidies ($p < 0.05$). An asterisk (*) denotes a significant difference between pre-stress and post-stress fish ($p < 0.05$). A plus sign (+) denotes a significant difference between acclimation temperatures ($p < 0.05$). A click (ǂ) denotes a significant temperature by stress interaction ($p < 0.05$). A filled circle (•) signifies a significant ploidy by temperature interaction ($p < 0.05$). Different letters indicate differences between treatment groups when there was a significant interaction term ($p < 0.05$).

sturgeon were significantly heavier than triploids ($F_{1,190} = 22.22$, $p < .001$) with a mean and s.d. of 498 ± 96 g (versus 440 ± 72 g for triploids). Diploid and triploid white sturgeon did not differ in fork length ($F_{1,64} = 0.32$, $p = .57$). Mean and s.d. of fork length was 41.4 ± 2.3 cm for diploid sturgeon and 41.2 ± 1.9 cm for triploid sturgeon.

Exposure to a netting stress resulted in significantly elevated plasma cortisol levels in diploid and triploid white sturgeon across different recovery times post-stress ($F_{1,24} = 8.89$, $p < .001$, Fig. 4). Ploidies did not differ in the kinetics of the cortisol response to an acute netting stressor ($F_{1,24} = 0.20$, $p = .66$, Fig. 4). Plasma cortisol levels were highest at 15 min post-netting stress and were similar to baseline (Time 0) levels by 60 min post-netting stress in both diploid and triploid sturgeon (Fig. 4).

4. Discussion

We investigated differences in stress response mechanisms of triploid and diploid white sturgeon to both warm temperature (22 °C) acclimation as well as acute stressors to better understand whether sensitivity to stress differs between 8 N and 12 N sturgeon. Triploid fish have 50% more chromosome copies, resulting in an increase in cell size, ultimately affecting cellular surface area to volume ratios (SA:V) (Small and Benfey, 1987). While many aspects of an organism's biology such as diffusion, signal transduction, and metabolic rate, are directly impacted by SA:V, our results suggest that some physiological parameters may be influenced by SA:V (i.e. metabolic enzymes); however, other physiological aspects, like the neuroendocrine stress response, do not appear to be impacted by SA:V (i.e. cortisol and glucose). The results suggest

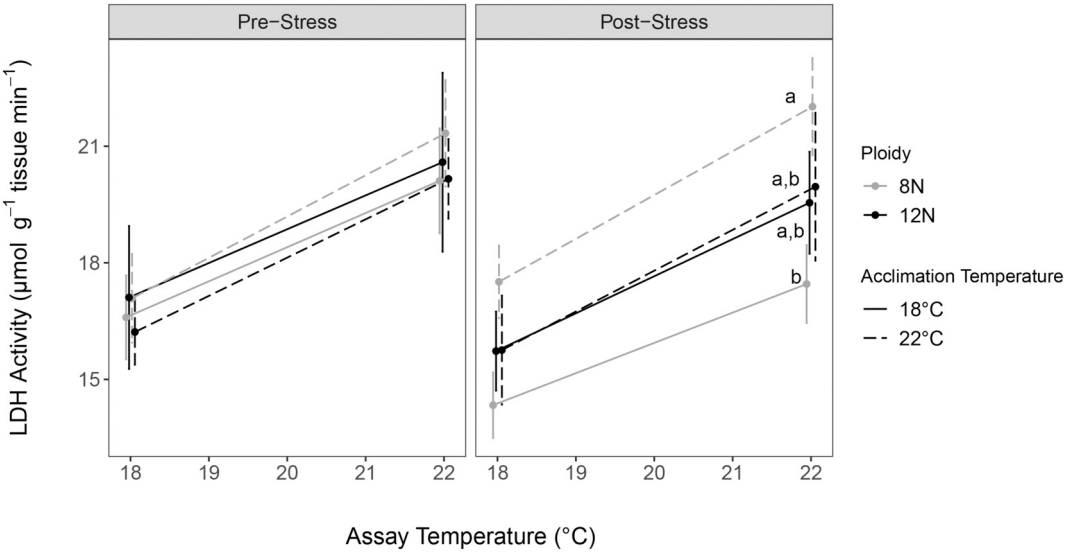

Fig. 2. Lactate dehydrogenase (LDH) activities (µmol min^{-1} g tissue^{-1}) of 8N (grey) and 12N (black) white sturgeon before and 30 minutes after a 10-minute water reduction stress following a 6-week temperature acclimation to either 18 °C (solid line) or 22 °C (dashed line) (n = 10 for each group). All homogenates were assayed at both 18 °C and 22 °C (x-axis). A three-way ANOVA was used to determine significant differences between treatments. There was a significant interaction between acclimation temperature and ploidy ($p < 0.05$); therefore, all levels of acclimation temperature and ploidy were compared using a Tukey test. Different letters denote significant differences across levels of acclimation temperature and ploidy ($p < 0.05$), at the 22 °C assay temperature.

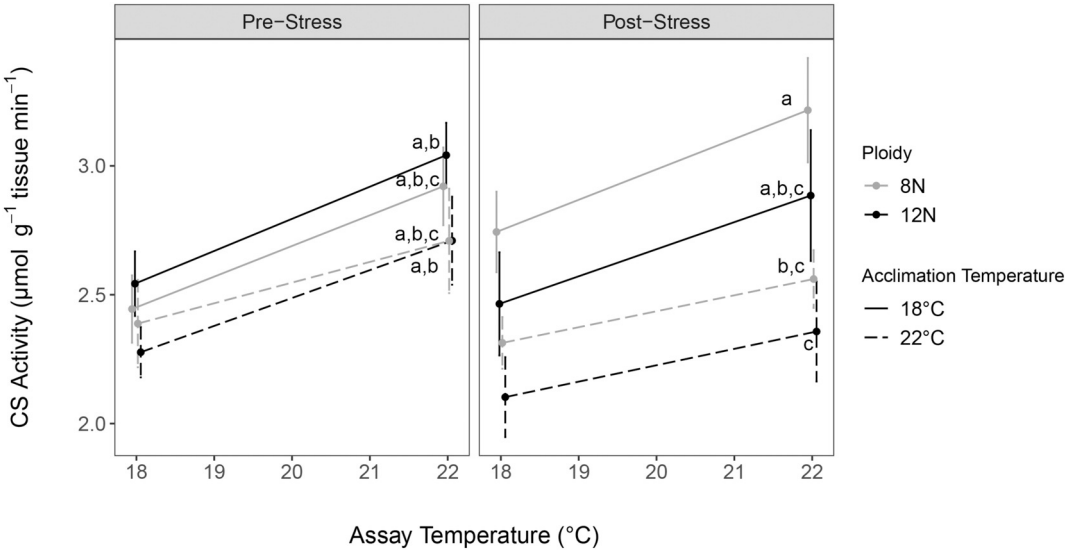

Fig. 3. Citrate synthase (CS) activities (μmol min^{-1} g $tissue^{-1}$) of 8N (grey) and 12N (black) white sturgeon before and 30 minutes after a 10-minute water reduction stress following a 6-week temperature acclimation to either 18 °C (solid line) or 22 °C (dashed line) (n = 10 for each group). All homogenates were assayed at both 18 °C and 22 °C (x-axis). A three-way ANOVA was used to determine significant differences between treatments. There was a significant main effect of acclimation temperature ($p < 0.05$) and a significant interaction between stress and ploidy ($p < 0.05$); therefore, all levels of acclimation temperature, ploidy, and stress treatments were compared using a Tukey test. Different letters denote significant differences across levels of acclimation temperature, ploidy, and stress ($p < 0.05$), at the 22 °C assay temperature.

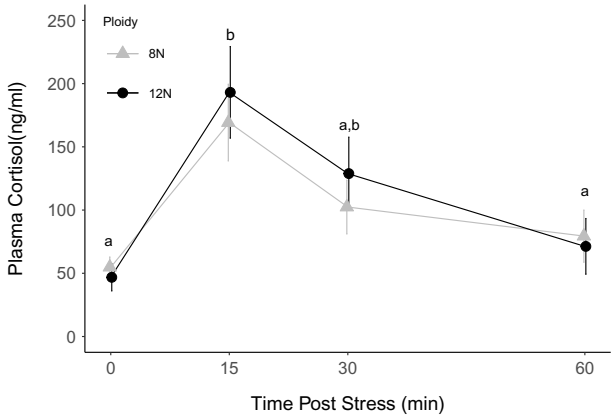

Fig. 4. Plasma cortisol concentrations (ng/ml) of 8 N (grey) and 12 N (black) white sturgeon prior to (Time 0) and 15, 30, and 60 min after exposure to a 5-min netting stress ($n = 4$ replicate tanks per ploidy and sampling time, with 6 fish/tank). Different letters denote significant differences between sampling times ($p < 0.05$).

that diploid and triploid white sturgeon have similar primary and secondary stress responses to an acute stressor; however, there were differences in metabolic enzyme activities after exposure to an acute stressor and warm acclimation between ploidies, suggesting differences in cellular energy metabolism. Comparisons of hematological parameters provide evidence of a reduced oxygen carrying capacity in triploid white sturgeon, which may contribute to the differences in cellular metabolism. Morphometric data from the acclimation trial (i.e. Experiment I) demonstrated reduced growth rates of triploid white sturgeon compared to diploids.

4.1. Acute stress following warm acclimation

Triploidy may impair the capacity for growth in white sturgeon, especially when acclimated to an elevated temperature. Over the six-week temperature acclimation period, the specific growth rates (SGR) of both ploidies were significantly lower when acclimated to a warmer water temperature (22 °C) compared to the ambient control temperature (18 °C, Table 1). Additionally, SGR in triploid white sturgeon was significantly reduced compared to diploids; in fact, triploid sturgeon in both acclimation temperatures demonstrated negative SGR (Table 1). A lowered SGR after long-term exposure to elevated temperatures suggests that the higher acclimation temperature was suboptimal for performance in white sturgeon, particularly triploid sturgeon. Differences in fish mortality (Ojolick et al., 1995), standard metabolic rate (Atkins and Benfey, 2008), and feed intake (Sambraus et al., 2017) indicate that triploid salmonids have a lower thermal optimum than their diploid counterparts. While it is a concern that triploid sturgeon exhibited negative growth rates over the duration of the experiment, both ploidies were fed the same rate, optimized for the 22 °C-acclimation temperature to allow for optimal growth rates. This was clearly not the case unfortunately as triploid sturgeon demonstrated negative growth. The feed rate used in the experiment was based on feeding tables that were established for high production aquaculture operations, rather than small research tanks/facilities. Additionally, the fish used in this experiment were switched from a satiation-based diet (before experimentation) to a restricted diet (during experimentation), which may have contributed to the poor growth rates. The feed rate chosen may not have been optimal for either ploidy (as diploids had a reduced growth rate at 22 °C vs 18 °C acclimation), yet there was no indication that any fish were unhealthy (i.e. no uneaten food or signs of morbidity). The lower SGR found in triploid white sturgeon is still particularly noteworthy and warrants further investigation.

Inherent differences in cell size and number impact several hematological parameters in triploid white sturgeon (Table 2). Of note is the lower concentration in total hemoglobin found in triploid sturgeon. It appears triploid sturgeon have some mechanism of compensating for a reduced number of erythrocytes (RBC count) by increasing the amount of hemoglobin per red blood cell (MEH) compared to diploids; however, this compensation does not appear to be sufficient to match diploid sturgeon total hemoglobin levels. The hemoglobin concentration per unit volume of red blood cells (MEHC) was similar between ploidies but the overall volume of packed red blood cells (Hct) was lower in triploid sturgeon, resulting in a significantly lower total hemoglobin concentration in triploid white sturgeon compared to diploids. These

results differ from comparisons made between 8 N and 10 N white sturgeon, where 10 N sturgeon had total hemoglobin concentrations compared to diploids (Leal et al., 2018). For most hematological parameters, diploid and triploid white sturgeon demonstrated similar responses to temperature acclimation; however, MEH increased with warm acclimation in triploid sturgeon but was unaffected by warm acclimation in diploid sturgeon. The significant effect of warm acclimation on MEH of white sturgeon triploids might be due to a slight increase in erythrocyte volume (MEV; Table 2). For fish acclimated to 22 °C, it appears triploid sturgeon have larger cells that contain more hemoglobin compared to diploids (i.e. can transport more O_2 per cell), yet the amount of hemoglobin per unit of volume is still the same as that of diploids (i.e. can transport the same amount of O_2 per unit volume of blood) due to triploids having fewer erythrocytes. The total hemoglobin is different between ploidies, because of a reduced hematocrit, suggesting that triploid white sturgeon, despite having larger cells, may have a lowered total oxygen carrying capacity than diploid white sturgeon.

Overall, both diploid and triploid white sturgeon appear to respond similarly when subjected to an acute water reduction stressor, providing evidence that a reduced SA:V does not affect primary and secondary physiological stress responses. Additionally, when acute water reduction stressor was preceded by a 6-week acclimation to elevated temperatures (22 °C), both diploid and triploid white sturgeon demonstrated a reduced ability to mount a typical acute stress response. Previous studies have shown that Adriatic sturgeon (*Acipenser naccarii*) acclimated to elevated temperatures demonstrated elevated resting plasma cortisol concentrations (Cataldi et al., 1998), and plasma lactate concentrations were higher in green sturgeon (*Acipenser medirostris*) maintained at a higher temperature (Lankford et al., 2003). Coping with chronic temperature stress to maintain homeostasis is an energetically demanding process in ectotherms (Wendelaar Bonga, 1997; Van Weerd and Komen, 1998). Similarly, the neuroendocrine stress response to an acute stressor aids in elevating blood glucose and oxygen levels to supply energy for a “fight or flight” response (Wendelaar Bonga, 1997). Both chronic and acute stress can deplete energy stores; therefore, chronic stress may leave an organism with less available energy to cope with a subsequent acute stressor (Sokolova et al., 2012). In our study, hematocrit, hemoglobin, and total gill protein increased with exposure to acute stress in diploid and triploid white sturgeon acclimated to 18 °C, demonstrating sufficient available energy to mount a typical primary and secondary stress response. In diploid and triploid sturgeon acclimated to 22 °C, if energy was allocated toward basal maintenance (i.e. a higher routine metabolic rate) and away from aerobic scope functions, then sturgeon would have a reduced amount of energy available to mount a typical generalized stress response. This would explain why bioindicators of stress, such as hematocrit, hemoglobin, and total gill protein decreased with exposure to an acute stressor in white sturgeon maintained at 22 °C. Alternatively, the documented differences in the responses of sturgeon may be due to differences in the kinetics of the response at different temperatures and therefore changes in the time to recorded peak levels of a particular bioindicator of stress. Multiple studies have found that warm acclimation decreased the time it took to reach recorded peak cortisol levels after exposure to an acute stressor (Barton and Schreck, 1987; Davis and Parker, 1990). The timing of our sampling may have been after hematocrit, hemoglobin, and total gill protein levels reached peak levels following acute stress in warm-acclimated sturgeon of both ploidies.

Comparisons of lactate dehydrogenase (LDH) activity, an anaerobic enzyme, in diploid and triploid white sturgeon gill tissue provide evidence of differences in the capacity to modulate cellular metabolic capacity after chronic exposure to elevated temperatures. In the current study, diploid white sturgeon demonstrated an increase in gill LDH activity with warm acclimation. In ectotherms, as temperature increases above its optimal temperature range, an organism will begin to rely more on anaerobic metabolism as oxygen becomes limited or as energy demand surpasses what can be supplied by aerobic mechanisms (see review by Pörtner, 2010). An increase in LDH specific activity after warm acclimation has been documented in many fishes (Shaklee et al., 1977; Mwangangi and Mutungi, 1994; Guderley et al., 2001; Leal et al., 2018) and may provide evidence that diploid sturgeon need to increase reliance on anaerobic mechanisms of energy production. In contrast, triploid sturgeon showed a slight decrease to no change in LDH activity (Fig. 2), suggesting that triploid white sturgeon could be limited in their ability to make long-term adjustments to LDH in response to warm acclimation. Alternatively, triploid sturgeon may not have needed to rely more heavily on anaerobic mechanisms of energy production at warmer temperatures, although branchial CS activity was not elevated to compensate for the additional energy demands of elevated temperature.

Diploid and triploid white sturgeon also differed in activity of a key aerobic enzyme, citrate synthase (CS), after being subjected to an acute water reduction stress, demonstrating a reduced capacity for short-term modification in triploid sturgeon after exposure to an acute stressor. Diploids exhibited an increase in CS activity while triploid sturgeon showed a reduction in CS activity after an acute water reduction stressor (Fig. 3). While there is a gap in the literature assessing the effect of acute stressors on aerobic metabolic enzyme activity, evidence suggests that cortisol increases CS activity, presumably to increase aerobic capacity when experiencing stress (Tripathi and Verma, 2003). Additionally, Leek et al. (2001) found that acute exercise (1 h) resulted in a significant increase in human skeletal muscle CS activity, suggesting an ability to upregulate enzyme activity when physiological energy demands increase. As diploid white sturgeon showed a general increase in CS activity after acute stress, it appears diploids exhibit a typical response to cortisol/stress in the alteration that occurred in CS activity. The lack of increase in CS activity in triploid white sturgeon in response to an acute water reduction stress may be an indication of a reduced capacity for short-term modulation of cellular aerobic metabolism enzyme activity. Differences in metabolic enzyme activities cannot merely be explained by differences in enzyme concentration, as total gill protein did not differ between ploidies (Table 2). Taken together, the differences in metabolic enzyme activities of LDH and CS provide initial evidence that triploid white sturgeon may be limited in their potential for metabolic reorganization following chronic (LDH) and acute (CS) stressors. Whether this is related to a reduced SA:V and cellular diffusion limitations in triploid sturgeon requires further examination.

4.2. Kinetics of the primary stress response

When exposed to a severe acute stressor (i.e. netting stress, Experiment II), diploid and triploid white sturgeon mounted a similar cortisol response. Both ploidies reached recorded peak cortisol levels in our study after 15 min of recovery and returned to pre-stress levels 60 min after netting. Other studies have reported similar timing of peak and recovery in plasma cortisol concentrations in diploid and triploid brook trout subjected to a 5-min handling/confinement stress (Biron and Benfey, 1994) and in Atlantic salmon after 2-h confinement stress (Sadler et al., 2000). Therefore, even when challenged with a more severe stressor (i.e. a higher recorded cortisol concentration compared to a water reduction stress), no marked differences in plasma cortisol concentrations between ploidies were found, further demonstrating diploid and triploid white sturgeon have similar primary stress responses to acute stressors.

4.3. Conclusions

Our study has demonstrated that diploid and triploid white sturgeon generally do not differ in their ability to cope with acute stressors, indicating that a reduction in SA:V does not limit signal transduction of

mediators of the primary and secondary stress responses in triploid sturgeon. Both ploidies have similar primary and secondary stress responses to long-term warming as well as a subsequent acute water reduction stress. In both ploidies, however, acclimation to an elevated temperature (22 °C) prevented some physiological parameters (hematocrit, hemoglobin, and total gill protein) from exhibiting a typical acute stress response. The results from Experiments I suggest differences in the growth rate between diploid and triploid white sturgeon; however, the cause of this difference is unclear and warrants further investigation. Diploid and triploid sturgeon did differ in their gill metabolic enzyme activities after both six weeks of warm acclimation (i.e. LDH activity) and after being subjected to an acute water reduction stress (i.e. CS activity). These variations in responses of metabolic enzyme activities may indicate an impaired ability to regulate cellular metabolic capacity in triploid white sturgeon as a mechanism to respond to environmental challenges. Since only the metabolic enzyme data seem to support diffusion limitation in triploid sturgeon, further investigation into the relationship between SA:V and energy metabolism in triploid sturgeon is needed to better understand the measured differences in capacity for long-term and short-term adjustments to metabolic enzyme activity. More research is needed to gain insight into whether this difference in cellular metabolic capacity hinders the whole animal metabolic rate and aerobic scope of triploid sturgeon.

Acknowledgements

This study was funded by the Western Regional Aquaculture Center (grant 2014-38500-22309) to AET and ADS from the USDA National Institute of Food and Agriculture and the Department of Animal Science, College of Agriculture and Environmental Science, and the California Agricultural Experimental Station of the University of California Davis (grant numbers CA-D-ASC-2252-H and CA-D-ASC-2253-RR to AET). MJL was supported by a UC Davis Eugene Cota-Robles Fellowship. We would like to thank Dennis Cocherell, Chessie Cooley-Rieders, Linda Deanovic and Matthew Stone at the Center for Aquatic Biology and Aquaculture at UCD for their assistance in our project.

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