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The effects of warm temperature acclimation on constitutive stress, immunity, and metabolism in white sturgeon (*Acipenser transmontanus*) of different ploidies

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ABSTRACT

Previous studies suggest fish with additional copies of their genome (polyploids) underperform in suboptimal conditions and may be more susceptible to stress and disease. The objective of this study was to determine the role ploidy plays in the physiological response of white sturgeon to chronically elevated water temperatures. White sturgeon of two ploidies (8 N and 10 N) were acclimated to ambient (18 °C) and warm (22 °C) water. Bioindices of stress (plasma cortisol, glucose and lactate, total erythrocyte count, hematocrit, hemoglobin, mean erythrocyte volume, mean erythrocyte hemoglobin, and mean erythrocyte hemoglobin concentration), immunity (respiratory burst, plasma lysozyme, and total leukocyte count), and cellular metabolic capacity (lactate dehydrogenase and citrate synthase activity) were measured before and after a 6-week acclimation period. Both ploidies appear comparable in their constitutive immune and stress parameters and respond similarly to warming. Hematological indices suggest 8 N and 10 N sturgeon are similar in oxygen carrying capacity; however, differences in enzyme activity between ploidies indicate that 10 N sturgeon may have a lower cellular aerobic capacity. Our results have implications for the screening and management of ploidy on white sturgeon farms and hatcheries, as the differences between ploidies may affect 10 N sturgeon performance at elevated water temperatures. Further research is needed to elucidate the differences in inducible stress and immune responses and metabolism of white sturgeon of different ploidies.

1. Introduction

Polyploids are organisms that have more chromosome sets than would be expected for that species. Polyploidy can occur spontaneously in wild fish populations, and can also be induced at fish hatcheries and farms by exposing newly fertilized eggs to a temperature, pressure, or chemical shock (Piferrer et al., 2009). At aquaculture facilities, there is interest in manipulating ploidy to achieve sterility to increase growth rates and reduce mating with wild stocks (Benfey, 2001). As the interest in raising polyploid fish on farms expands with new species, the need to understand physiological differences in polyploid organisms becomes increasingly pertinent. The physiology of diploid and triploid salmonids has been well studied (Benfey, 1999). Diploid and triploid physiology has been compared in brook trout (Hyndman et al., 2003), rainbow trout (Scott et al., 2015), Coho salmon (Small and Benfey, 1987), Atlantic salmon and brook charr (Atkins and Benfey, 2008); however, little is known about physiological differences between various ploidies in sturgeon species. To our knowledge, only one study has assessed physiological parameters of sturgeon of different ploidy levels (Beyea et al., 2005).

A key difference among polyploid organisms is cell size, number, and therefore, cellular surface area to volume ratios (Benfey, 1999). Cell surface area to volume ratios are related directly to cellular functions, such as diffusion and membrane transport. Since most biological processes rely on diffusion and membrane transport, changes in these cellular functions can have physiological ramifications that may translate to differences in whole animal performance. A larger cell size could potentially increase the distance required for signal transduction from cell surface to nucleus, thereby altering transcription and translation (Wu et al., 2010). Changes to transcription and translation can impact key physiological processes, such as stress, immunity and metabolism. Differences in the stress and immune responses as well as metabolic processes in polyploid organisms are important considerations from a scientific and an applied viewpoint as stress and immune

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function are important aspects of growth, reproduction, and disease resistance (Mommsen et al., 1999).

Studies assessing the stress response of diploid and triploid salmonids have primarily focused on the response to acute stress (i.e. handling or confinement) and have found that both constitutive and induced levels of plasma cortisol are similar between ploidies (Biron and Benfey, 1994; Benfey and Biron, 2000; Sadler et al., 2000a). Compared to diploids, triploids, however, are often described as performing poorly under suboptimal conditions, such as elevated temperatures (Ojolick et al., 1995; Altimiras et al., 2002; Hyndman et al., 2003; Atkins and Benfey, 2008) and hypoxic environments (e.g. Ellis et al., 2013; Hansen et al., 2015). Little is known about the stress responses of fishes of different ploidies to chronic stressors, particularly chronic elevated temperatures. One of the main environmental drivers of global climate change is increasing water temperatures (IPCC, 2014), and in ectotherms, water temperature has a pervasive effect on organismal biochemistry and physiology (Brett, 1971). Acclimation to increased water temperatures involves physiological adjustments like changes in hematology and metabolic activity (Houston, 1980), all of which may be impacted by ploidy due to a difference in cell size and number. Additionally, biological processes, including immune function and metabolism, are heavily influenced by water temperature in ectotherms.

The effect of elevated water temperature on the immune system of fishes has been well studied. Typically, an increase in water temperature results in an increase of general immune parameters in fishes (reviewed by Bowden, 2008). Yet, other studies have shown that expression of innate and adaptive immune genes is highest at intermediate temperatures, suggesting an optimal temperature range for immune function (Pérez-Casanova et al., 2008). To date, no studies have assessed white sturgeon innate immunity either constitutively or in response to chronically elevated water temperature. Additionally, no studies have assessed whether ploidy affects the immune system alterations that occur when exposed to elevated temperatures. Studying immunity in fish of different ploidies is pertinent, as having fewer cells, specifically immune cells, has the potential to impact an organism's constitutive immunity.

Metabolic activity is also directly impacted by environmental temperature in ectotherms and provides insight into the 'cost of living' of an organism (Shaklee et al., 1977). As fish acclimate to different temperatures, reorganization of mitochondria, changes in metabolic rate and cellular metabolic pathways, and alterations in energy stores can occur (Johnston and Dunn, 1987). To date, little is known about the differences in cellular metabolic capacity between diploid and triploid fish and whether the metabolic capacity to acclimate to warmer temperatures are similar between fish of different ploidies.

The white sturgeon (*Acipenser transmontanus*) is a popular recreational fish species along the Pacific coast and is also an important aquaculture species, specifically in the caviar industry. White sturgeon have a unique ploidy compared to salmonids, having undergone multiple genome duplications throughout their evolution, such that the typical ploidy is 8 N (i.e. having eight sets of chromosomes). Spontaneous autopolyploidy has occurred on both farms and hatcheries in white sturgeon, resulting in 12 N fish (referred to as triploid) (Drauch Schreier et al., 2011). Triploid white sturgeon are fertile, a characteristic that is unique compared to salmonid triploids, which allows for intermediate ploidies (e.g. 10 N) between diploid and triploid that could not be found in salmonid species. The intermediate ploidies that are possible in white sturgeon provide an opportunity for the study of various levels of polyploidy that would otherwise not be possible in other fish species. The goal of this study was to determine if 10 N white sturgeon differ in their constitutive indices of stress, immunity, and metabolism under ambient and warming (+4 °C) conditions compared to 8 N sturgeon. An elevation in temperature of 4 °C was based on global climate change models, which predict an increase in water temperature between 2 and 5 °C by the end of the century (IPCC, 2014). The temperature range used in this experiment also reflects the range of temperatures juvenile sturgeon are exposed to on an annual basis (Van Eenennaam et al., 2004) and provides an indication of how 10 N sturgeon would perform in a culture setting compared to 8 N sturgeon. In assessing general stress, immune, and metabolic indices we sought to provide insight into the general health of the fish and help indicate if producers should be concerned with having intermediate ploidies on their farms. Based on previous studies demonstrating similarities between diploid and triploid salmonids in constitutive stress levels (Biron and Benfey, 1994; Benfey and Biron, 2000; Sadler et al., 2000a), immune parameters (Budiño et al., 2006), and metabolic status (Scott et al., 2015), we expected 10 N sturgeon to have a similar constitutive stress, immune, and metabolic parameters to 8 N sturgeon at ambient temperature (control). However, we predicted that the response to warming would not be similar between ploidies because evidence suggests that triploid salmonids underperform when acclimated to elevated water temperatures (Ojolick et al., 1995; Altimiras et al., 2002; Hyndman et al., 2003; Atkins and Benfey, 2008). We predicted that after warm water acclimation 10 N white sturgeon would demonstrate elevated constitutive stress and metabolic responses, and reduced innate immune capabilities compared to 8 N white sturgeon.

2. Materials and methods

2.1. Fish source and husbandry

Ovulated eggs from two white sturgeon females (one 8 N and one 12 N) and milt from one male (8 N) were acquired from domesticated broodstock at a northern California sturgeon farm to produce 8 N and 10 N half-siblings. Stock originated from Sacramento River wild-caught parents, and both ploidy broodstock used for this experiment were similar F2 generation domesticated. Broodstock were reared at 18–22 °C until mature, and then they were maintained and spawned at 10–15.5 °C. Ploidy for these broodstock was verified using flow cytometry following Schreier et al. (2013).

Eggs covered in ovarian fluid were kept in oxygen filled bags in an ice chest at 15.5 ± 1.0 °C, and milt was kept in an oxygenated bag in an ice chest, on wet ice. Both ice chests were transported to the Putah Creek Hatchery Facility at the University of California, Davis, CA, USA. Eggs from each female were crossed with the same male using standard fertilization and de-adhesion procedures (Van Eenennaam et al., 2004) and incubated at 15.5 ± 0.5 °C in a flow-through hatchery system at the Putah Creek Hatchery Facility. After hatch, larvae were transported to the Center for Aquatic Biology and Aquaculture (on the UC Davis campus) where they remained for the duration of the rearing period and during experimentation. After transportation, larvae were switched to a flow-through tank system with well water at 18.5 ± 0.5 °C. Both families (8 N and 10 N) were reared under similar conditions until experimentation as juvenile fish (age 14.5 months, mean weight 0.90 kg and 1.22 kg for 8 N and 10 N fish, respectively).

After yolk-sac depletion, larvae were initially fed daily, ad libitum, using a 24-h belt feeder and a semi-moist diet (Rangen Inc., Buhl, ID; 45% protein, 18% fat). At approximately 2 months post hatch (approximately 5 g) fish were weaned onto extruded sinking classic trout diet (Skretting USA,Tooele, Utah; 40% protein, 12% fat), and feed rates gradually decreased from approximately 6% to 1%, as the fish grew. Husbandry, handling, and sampling procedures followed the protocol approved by the UC Davis Institutional Animal Care and Use Committee (protocol #18329).

2.2. Experimental design

Prior to the start of the temperature acclimation, 24 white sturgeon juveniles from each ploidy were tagged using a passive integrated transponder (PIT) tag. 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.

After tagging, fork length and body weight were recorded. Sturgeon were then placed in one of two tanks, separated by ploidy, and allowed to recover for 12 days.

After the recovery period, six sturgeon from each ploidy were randomly assigned to one of two temperature acclimation treatments (2 replicate tanks/treatment), with a total of 12 sturgeon in each tank. Control-temperature acclimation tanks were maintained at a temperature of 18.6 ± 0.2 °C (ambient temperature of well water) and warm temperature acclimation tanks were maintained at a temperature of 22.1 ± 0.6 °C using an external heater. Temperature was recorded every 15 min by HOBO® temperature loggers (Onset Computer Corporation, Bourne, USA) that remained in the tank through the duration of the experiment. Sturgeon were fed a basic trout 5.0 mm pelleted diet at 0.74% of body weight (Skretting USA, Tooele, Utah; 40% protein, 12% fat) using 24-h automatic belt feeders. A feed rate of 0.74% body weight was based on fish size and water temperatures (22 °C) from established aquaculture guidelines for white sturgeon (Van Eenennaam et al., 2004). All tanks were fed the same ration based on the elevated temperature to ensure feed was not a limiting factor in the warm-acclimated fish. Sturgeon were held in fiberglass tanks (122 cm diameter, 42 cm height, 490 l), each with its own air stone, supplied with degassed well-water (flow rate of 0.365 ± 0.006 l/s). Sturgeon were held in the temperature acclimation treatments for six weeks prior to sampling.

2.3. Blood and tissue sampling

To obtain pre-acclimation levels (Time 0) of constitutive stress and immune parameters for both 8 N and 10 N white sturgeon, six sturgeon from each ploidy were sampled on a single day at the start of the temperature acclimation experiment. Following the six-week acclimation period, all sturgeon were sampled from the temperature acclimation treatments over a two-day period. One replicate tank from the warm-acclimation treatment and one replicate tank from the control-acclimation group were sampled on each day in the morning to control for hormonal changes due to circadian rhythms. Sturgeon were fasted for 24 h prior to sampling. During sampling, sturgeon were netted and immediately placed in a lethal dose of buffered MS-222 (1000 mg/l). Blood was drawn from the caudal vein using 20 gauge needles and collected in heparinized vacutainers. For each tank, sturgeon were exposed to MS-222 and blood was drawn from all 12 fish within 6 min. Blood was immediately processed and aliquoted for the different analyses requiring whole blood (Hct, RBC, WBC, and Hb; details below). A 500 µl aliquot of blood was centrifuged at 12,000 ×*g* for 5 min. Plasma was aliquoted into fresh microcentrifuge tubes and immediately frozen on dry ice and stored at −80 °C for future analysis. The remaining whole blood was stored on ice for respiratory burst analysis. After blood was drawn, fish weight, fork length, and PIT tag number were recorded. Gill tissue was dissected, and the sex was determined for each sturgeon, when possible. Specific growth rate (SGR) was calculated using the following equation:

$$\mathrm{SGR}(\%/\mathrm{d}) = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{time (d)}} \times 100$$

2.4. Measuring the generalized stress response

For total erythrocyte counts (RBC), 5 µl of whole blood was pipetted into 995 µl of Hendricks' solution (Hendricks, 1952) and kept refrigerated until future analysis. Counts were taken using a hemocytometer. For each chamber, erythrocytes were counted in five 0.2 mm^2 area squares and the average of the two chambers was taken. Hematocrit (Hct) was determined using 75 mm capillary tubes that were centrifuged for 3 min at 11000*g* in duplicate for each sample. Hemoglobin (Hb) was measured spectrophotometrically in triplicate at 540 nm using a cyanmethemoglobin kit following manufacturer's instructions (Teco Diagnostics, Anaheim, USA). Plasma cortisol was analyzed spectrophotometrically in duplicate at 650 nm using a competitive binding enzyme-linked immunosorbent assay (ELISA) kit following manufacturer's instructions (Neogen Corporation, Lansing, USA). The ELISA kit was validated for white sturgeon plasma using a standard spike recovery protocol and tested for linearity (Barry et al., 1993). Mean intra- and inter-assay coefficients of variation were 4.7 and 13.1% respectively. The spike recovery assay yielded a mean recovery of 103.9%, and the test for linearity with serial dilutions resulted in a mean R^2 value of 0.98. Plasma glucose and lactate were evaluated using a YSI 2700 Bioanalyzer (YSI Incorporated, Yellow Springs, USA).

Hb, Hct, RBC values were used to calculate mean erythrocyte volume (MEV), mean erythrocytic hemoglobin concentration (MEH), and mean erythrocytic hemoglobin content (MEHC) (Houston, 1990). The following equations were used:

$$\mathrm{MEV\ (fl/cell)} = \frac{\mathrm{Hct\ (\%)} * 1000}{\text{Total RBC } (10^6 \text{ cells}/\mu\mathrm{l})}$$

$$\mathrm{MEH\ (pg/cell)} = \frac{\mathrm{Hb\ (g/l)}}{\text{Total RBC } (10^6 \text{ cells}/\mu\mathrm{l})}$$

$$\mathrm{MEHC\ (g/dl)} = \frac{\mathrm{Hb\ (g/dl)}}{\mathrm{Hct\ (\%)}}$$

2.5. Measuring the innate immune response

Respiratory burst activity was assessed using a glass-adherent cell protocol modified using a hemocytometer (Anderson et al., 1992; Ackerman et al., 2006). Whole blood was collected in 75 mm capillary tubes and centrifuged at 11,000 ×*g* for 3 min. Capillary tubes were then cut below the buffy coat (i.e. leukocytes and platelets) and expelled into individual tubes. Three µl of vortexed plasma/buffy coat solution was pipetted onto a coverslip, which was then placed in a moist chamber (Petri dish with moistened filter paper) and incubated at room temperature for 30 min. After incubation, coverslips were gently washed with phosphate buffered saline (pH 7.4) and placed on a hemocytometer. Nitro blue tetrazolium (NBT, 10 µl of 1 mg/ml) was added to each chamber and incubated at room temperature for an additional 30 min. Cells containing a blue/purple halo were considered NBT positive. The number of positive and negative cells containing morphological features of monocytes and neutrophils were counted in a 1 mm^2 area and expressed as a percentage. Both chambers of the hemocytometer were counted and averaged together. Respiratory burst activity was not measured in samples collected before acclimation.

Plasma lysozyme was determined spectrophotometrically in quadruplicate using a microplate assay as described by Ackerman et al. (2006). Briefly, 10 µl of diluted plasma or hen egg white lysozyme (HEWL) standards and 250 µl of *Micrococcus lysodeikticus* solution (0.025% *w/v*) in phosphate buffered saline (60 mM, 6.2 pH) were added to each well. The change in optical density at 450 nm over 20 min was expressed as hen egg white lysozyme (HEWL) equivalents.

For total leukocyte counts, 5 µl of whole blood was pipetted into 995 µl of Hendricks' solution (Hendricks, 1952) and kept refrigerated until future analysis. Counts were taken using a hemocytometer. For each chamber, leukocytes were counted in four different 1 mm × 1 mm squares and the average of the two chambers was taken.

2.6. Measuring metabolic enzyme activity

Lactate dehydrogenase (LDH) and citrate synthase (CS) enzyme assays were measured as previously described by Hansen and Sidell (1983), optimized for white sturgeon gill tissue using the same homogenization buffer. Briefly, approximately 80–150 mg of gill tissue was homogenized in ice cold potassium phosphate buffer (50 mM, pH 7.5) using a handheld homogenizer (PRO Scientific Inc., Oxford, USA). The

homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant was transferred to a new tube and used for enzyme analyses. The activity of LDH was determined by monitoring the conversion of NADH to NAD^+ spectrophotometrically (Biotek Synergy HT, Winooski, VT, USA) at 340 nm in imidazole buffer (52.5 mM, pH 7.5) with 2.64 mM pyruvate in triplicate. 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 in triplicate. All homogenates were run at both 18 °C and 22 °C to match experimental treatments. Temperature coefficient (Q_{10}) values were calculated as the ratio of the activity at 22 °C to the activity at 18 °C raised to the power of 10 over the difference in temperatures (4 °C). Metabolic enzyme activities were not measured in samples collected before acclimation.

2.7. Statistical analysis

Data were analyzed with R (http://www.R-project.org) using the RStudio interface (v 3.3.0). Statistical analyses were performed on pre-acclimation (Time 0) and post-acclimation data separately. For all statistical tests, an α of 0.05 was used as the significance level. Differences between ploidies for pre-acclimation data were assessed using a two-tailed, two-sample *t*-test. Statistical relationships for data following temperature acclimation were analyzed using a two-way analysis of variance (ANOVA) with acclimation temperature and ploidy as fixed factors. Due to limitations on temperature control, only two replicate tanks per temperature were available for use in this experiment. 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 two-way analysis of covariance (ANCOVA) was used. Prior to running any *t*-test, ANOVA, or ANCOVA, the assumptions of normality and homogeneity of variance of residuals were tested statistically using a Shapiro-Wilk and Levene's Test, respectively, and visually inspected using a Q-Q plot and a fitted vs. residuals plot. When normality was violated, a log transformation was applied to the response variable. When homogeneity of variance within levels was violated in an ANOVA or ANCOVA, a generalized least squares model with a "varIdent" variance structure was used ("nlme" package; Pinheiro et al., 2016). When homogeneity of variance was violated in a *t*-test, a Welch Two Sample *t*-test was used. All values presented are mean ± 1 s.d. Differences between treatments were determined using a Tukey's post hoc test ("lsmeans" package; Lenth, 2016).

A principal component analysis (PCA) was performed to determine which variables were most correlated to one another and which variables accounted for the most variation within the data. Total erythrocyte counts, hematocrit, hemoglobin, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, plasma cortisol, glucose and lactate, respiratory burst activity, plasma lysozyme activity, total leukocyte counts, lactate dehydrogenase activity and Q_{10}, citrate synthase activity and Q_{10}, weight, and fork length were all included in the PCA as a source of variation. The PCA was performed using scaled values and the prcomp function. A contributions plot and biplots were created using the "factoextra" (Kassambara and Mundt, 2017) and "ggbiplot" (Vu, 2011) packages respectively.

3. Results

3.1. Morphometric data

In general, 10 N sturgeon were larger than 8 N sturgeon and warm acclimated sturgeon were smaller than those at the ambient acclimation temperature (Table 1). The mean pre-acclimation weight was 28.7% greater for 10 N sturgeon than for 8 N sturgeon (t = 3.49, df = 46, p-value = 0.001). After acclimation, significant differences were found between ploidies ($F_{1,40} = 29.07$, $p = 3.4 \times 10^{-6}$) and acclimation temperatures ($F_{1,40} = 7.36$, $p = 9.8 \times 10^{-3}$), with 10 N sturgeon acclimated to 22 °C having the largest mean weight (Table 1). Specific growth rate (SGR) was significantly higher for 10 N sturgeon ($F_{1,40} = 12.725$, $p = 9.54 \times 10^{-4}$), growing an average of 0.47% per day (vs 0.19% per day for 8 N sturgeon); SGR was not affected by acclimation temperature ($F_{1,40} = 2.47$, p = 0.12). Pre- and post-acclimation fork length followed a similar trend to pre- and post-acclimation weight. Before acclimation, 10 N sturgeon were, on average, 11.3% longer than 8 N sturgeon (t = 5.38, df = 46, p-value = 2.5×10^{-6}). After acclimation, both ploidy ($F_{1,40} = 33.75$, $p = 8.8 \times 10^{-7}$) and acclimation temperature ($F_{1,40} = 4.09$, p = 0.049) significantly affected fork length, again with 10 N sturgeon acclimated to 22 °C having the longest mean fork length (Table 1). There were no significant interactions between ploidy and acclimation temperature on any morphometric data.

Table 1
Morphometric data of 8 N and 10 N white sturgeon before and after a 6-week temperature acclimation (n = 12). A dagger (†) denotes a significant difference between ploidies ($p < 0.05$). A plus sign (+) denotes a significant difference between acclimation temperatures ($p < 0.05$).

	8 N		10 N	
	18 °C Acclimated	22 °C Acclimated	18 °C Acclimated	22 °C Acclimated
Pre-Acclimation Weight (kg)†	0.90 ± 0.17	0.84 ± 0.21	1.22 ± 0.29	1.02 ± 0.29
Post-Acclimation Weight (kg)†,+	1.00 ± 0.19	0.88 ± 0.23	1.51 ± 0.33	1.21 ± 0.30
Pre-Acclimation Fork Length (cm)†	48.3 ± 4.0	46.8 ± 4.1	54.0 ± 2.9	51.8 ± 4.3
Post-Acclimation Fork Length (cm)†,+	50.9 ± 4.0	49.5 ± 5.1	58.4 ± 3.1	55.2 ± 4.2
Specific Growth Rate (%/day)†	0.27 ± 0.15	0.11 ± 0.28	0.52 ± 0.32	0.42 ± 0.32

3.2. Pre-acclimation indices of constitutive stress and immune parameters

Generally, 8 N and 10 N sturgeon exhibited similar constitutive stress and immune parameters, but did differ in total erythrocyte counts, hematocrit, and plasma lactate levels. Compared to 8 N sturgeon, 10 N sturgeon had a significant reduction in erythrocytes by 25.0% (t = −2.34, df = 10, p = 0.041) and a mean hematocrit that was 14.8% lower (t = −2.26, df = 10, p = 0.047). Ploidy did not significantly affect hemoglobin (t = 1.26, df = 10, p = 0.24), mean erythrocyte volume (MEV, t = 0.42, df = 10, p = 0.68), mean erythrocyte hemoglobin (MEH, t = 0.65, df = 10, p = 0.53), or mean erythrocyte hemoglobin concentration (MEHC, *t* = 0.40, df = 10, p = 0.69) in pre-acclimation sturgeon (Table 2). Ploidy did not have a significant effect on plasma cortisol (t = −2.07, df = 5.15, p = 0.09) or plasma glucose (t = 1.67, df = 10, p = 0.13). Plasma lactate differed between ploidies (t = 4.34, df = 10, p = 0.001), with lower concentrations in 8 N sturgeon (0.63 ± 0.17 mmol/l), compared to 10 N sturgeon (1.13 ± 0.23 mmol/l). Lysozyme activity was not significantly affected by ploidy (t = 1.607, df = 10, p = 0.139). A lower total erythrocyte count was found in 10 N sturgeon ($4.3 \pm 0.6 \times 10^4$ cells/μl) compared to 8 N sturgeon ($5.6 \pm 1.3 \times 10^4$ cells/μl); however, this difference was not significant (t = −2.186, df = 10, p = 0.054).

3.3. Constitutive indices of stress following temperature-acclimation

Overall, 8 N sturgeon had more erythrocytes per volume of whole blood, higher hematocrit, and lower mean erythrocyte volume and

Table 2
Hematological parameters of 8 N and 10 N white sturgeon before (Time 0, N = 6) and after a 6-week temperature acclimation (n = 12). An asterisk (*) denotes a significant difference between ploidies at Time 0 ($p < 0.05$). A dagger (†) denotes a significant difference between ploidies after temperature acclimation ($p < 0.05$). A plus sign (+) denotes a significant difference between acclimation temperatures ($p < 0.05$). RBC = total erythrocyte counts, Hct = hematocrit, Hb = hemoglobin, MEV = mean erythrocyte volume, MEH = mean erythrocyte hemoglobin, MEHC = mean erythrocyte hemoglobin concentration.

	8 N			10 N		
	Time 0	18 °C Acclimated	22 °C Acclimated	Time 0	18 °C Acclimated	22 °C Acclimated
RBC (10^6 µl^{-1})$^{*,\dagger,+}$	0.92 ± 0.22	0.66 ± 0.10	0.74 ± 0.12	0.69 ± 0.11	0.49 ± 0.11	0.57 ± 0.09
Hct (%)$^{*,\dagger,+}$	27 ± 3	25 ± 2	28 ± 2	23 ± 3	23 ± 4	25 ± 2
Hb (g/dl)$^{+}$	6.3 ± 0.6	5.8 ± 0.9	6.4 ± 0.6	5.6 ± 1.2	5.3 ± 1.1	6.0 ± 0.6
MEV (fl/cell)†	319 ± 126	383 ± 38	388 ± 56	341 ± 31	474 ± 69	446 ± 64
MEH (pg/cell)†	73 ± 26	90 ± 16	87 ± 10	81 ± 14	109 ± 13	108 ± 13
MEHC (g/dl)	23.2 ± 1.3	23.3 ± 2.8	22.6 ± 2.5	23.8 ± 3.1	23.2 ± 3.4	24.3 ± 1.8

mean erythrocyte hemoglobin, when compared to 10 N sturgeon. Sturgeon acclimated to 22 °C had more erythrocytes and greater concentrations of hemoglobin per volume of whole blood and higher hematocrit, when compared to sturgeon acclimated to 18 °C (Table 2). In both temperature acclimation groups (18 °C and 22 °C), ploidy had a significant effect on total erythrocyte counts ($F_{1,40} = 31.57$, $p = 1.6 \times 10^{-6}$), hematocrit ($F_{1,39} = 7.84$, $p = 0.008$), but not on hemoglobin ($F_{1,40} = 2.96$, $p = 0.09$). On average, 10 N sturgeon had 24.4% fewer erythrocytes and a reduction in hematocrit by 18.7%, compared to 8 N sturgeon. MEV ($F_{1,40} = 21.08$, $p = 4.3 \times 10^{-5}$) and MEH ($F_{1,40} = 26.85$, $p = 6.6 \times 10^{-6}$) also differed by ploidy, while MEHC did not differ by ploidy ($F_{1,40} = 0.92$, $p = 0.34$). The mean MEV was 19.3% larger and the mean MEH was 22.6% higher for 10 N sturgeon, compared to 8 N sturgeon. Acclimation temperature had a significant effect on total erythrocyte counts ($F_{1,40} = 7.19$, $p = 0.01$), hematocrit ($F_{1,39} = 13.99$, $p = 5.9 \times 10^{-4}$), and hemoglobin ($F_{1,40} = 7.47$, $p = 0.009$), but not on MEV ($F_{1,40} = 0.48$, $p = 0.49$), MEH ($F_{1,40} = 0.25$, $p = 0.62$) or MEHC ($F_{1,40} = 0.09$, $p = 0.77$). None of the hematological indices had significant interactions between ploidy and acclimation temperature: total erythrocyte counts ($F_{1,39} = 0.005$, $p = 0.95$), hematocrit ($F_{1,39} = 1.12$, $p = 0.30$), hemoglobin ($F_{1,40} = 0.09$, $p = 0.77$), MEV ($F_{1,40} = 1.004$, $p = 0.32$), MEH ($F_{1,40} = 0.07$, $p = 0.80$), MEHC ($F_{1,40} = 1.49$, $p = 0.23$).

Plasma cortisol was significantly affected by ploidy ($F_{1,39} = 5.80$, $p = 0.02$) but not acclimation temperature ($F_{1,39} = 3.06$, $p = 0.09$), with no interaction between ploidy and acclimation temperature ($F_{1,39} = 0.15$, $p = 0.70$). Lower plasma cortisol levels were found in 10 N sturgeon (8.6 ± 7.0 ng/ml) compared to 8 N sturgeon (32.1 ± 48.7 ng/ml). Both before and after the acclimation period, 8 N sturgeon demonstrated high variability in cortisol levels when held at 18 °C (Fig. 1A). Plasma glucose was not significantly affected by ploidy ($F_{1,40} = 0.03$, $p = 0.86$) or by acclimation temperature ($F_{1,40} = 2.22$, $p = 0.14$; Fig. 1B). Like plasma glucose, plasma lactate also did not differ between ploidies ($F_{1,40} = 0.003$, $p = 0.96$) or between acclimation temperatures ($F_{1,40} = 0.003$, $p = 0.96$; Fig. 1C).

3.4. Constitutive indices of innate immune parameters following temperature-acclimation

Overall, across both acclimation temperatures, both ploidies were similar in constitutive innate immune parameters after the acclimation period. Respiratory burst activity was not significantly different between ploidies ($F_{1,40} = 0.01$, $p = 0.92$) or acclimation temperatures ($F_{1,40} = 0.25$, $p = 0.62$; Fig. 2A). Acclimation temperature had a significant effect on plasma lysozyme activity ($F_{1,40} = 6.67$, $p = 0.01$), with higher activity in sturgeon acclimated to 22 °C (12.4 ± 3.9 µg/ml HEWL) compared to sturgeon acclimated to 18 °C (9.9 ± 2.4 µg/mL HEWL; Fig. 3B). Ploidy did not have a significant effect on plasma lysozyme activity ($F_{1,40} = 0.98$, $p = 0.32$) and there was no interaction between ploidy and acclimation temperature ($F_{1,40} = 0.03$, $p = 0.87$). Leukocyte counts differed significantly by ploidy ($F_{1,40} = 16.39$, $p = 2.3 \times 10^{-4}$), with 10 N sturgeon having, on average, 21.4% fewer leukocytes than 8 N sturgeon (Fig. 2C). There was no significant effect of acclimation temperature on white blood cell counts ($F_{1,40} = 1.42$, $p = 0.24$) and no significant interaction between ploidy and acclimation temperature ($F_{1,40} = 1.56$, $p = 0.22$).

3.5. Metabolic enzyme activity following warm-acclimation

Overall, temperature affected both metabolic enzymes, and ploidies differed in their constitutive metabolic enzyme activity. Gill citrate synthase (CS) specific activity was significantly affected by ploidy ($F_{1,87} = 5.86$, $p = 0.02$), acclimation temperature ($F_{1,87} = 5.58$, $p = 0.02$) and assay temperature ($F_{1,87} = 12.61$, $p = 6.2 \times 10^{-4}$). In 8 N sturgeon, mean CS activity was 12.6% lower in warm-acclimated fish compared to ambient-acclimated fish, while 10 N sturgeon only had a reduction of 0.1% with warm acclimation. The interaction between ploidy and acclimation temperature was not significant ($F_{1,87} = 3.12$, $p = 0.08$). The highest CS activity was seen in 8 N sturgeon acclimated to 18 °C (Fig. 3A) and CS activity of 8 N sturgeon acclimated to 22 °C showed the greatest increase in activity when the assay temperature was increased by 4 °C. Gill lactate dehydrogenase (LDH) specific activity was significantly higher in sturgeon acclimated to 22 °C compared to those acclimated to 18 °C ($F_{1,86} = 7.57$, $p = 0.007$; Fig. 3B). The LDH activity of 10 N sturgeon was significantly higher when compared to 8 N sturgeon ($F_{1,86} = 6.58$, $p = 0.01$). There was no significant interaction between ploidy and acclimation temperature ($F_{1,86} = 0.06$, $p = 0.82$). LDH activity increased with increasing assay temperature ($F_{1,86} = 21.17$, $p = 1.4 \times 10^{-5}$).

Temperature quotient (Q_{10}) values for CS activity significantly differed by acclimation temperature ($F_{1,40} = 4.18$, $p = 0.048$) and that effect depended on ploidy (significant ploidy: temperature interaction; $F_{1,40} = 4.36$, $p = 0.043$; Table 3). There was no difference in Q_{10} values between acclimation temperatures for 10 N sturgeon; however, warm-acclimated 8 N sturgeon had a mean Q_{10} that was 12.6% higher than 8 N sturgeon acclimated to ambient water temperatures. The Q_{10} values for LDH activity were not significantly affected by ploidy ($F_{1,40} = 0.12$, $p = 0.73$) or temperature ($F_{1,40} = 1.24$, $p = 0.27$; Table 3).

The ratio of LDH to CS activity was significantly higher for 10 N sturgeon ($F_{1,79} = 12.74$, $p = 6 \times 10^{-4}$) and 22 °C-acclimated sturgeon ($F_{1,79} = 19.44$, $p = 3.2 \times 10^{-5}$), with no interaction between acclimation temperature and ploidy ($F_{1,79} = 0.02$, $p = 0.90$). On average, LDH:CS was 37.7% greater in 10 N sturgeon, and warm acclimation increased LDH:CS by 24.2%. Assay temperature did not significantly affect LDH:CS ratios ($F_{1,79} = 0.26$, $p = 0.61$; Table 3).

3.6. Principal component analysis

Using principal component analysis, we obtained two relevant principal components (PCs). The first and second PCs explained 24.1%

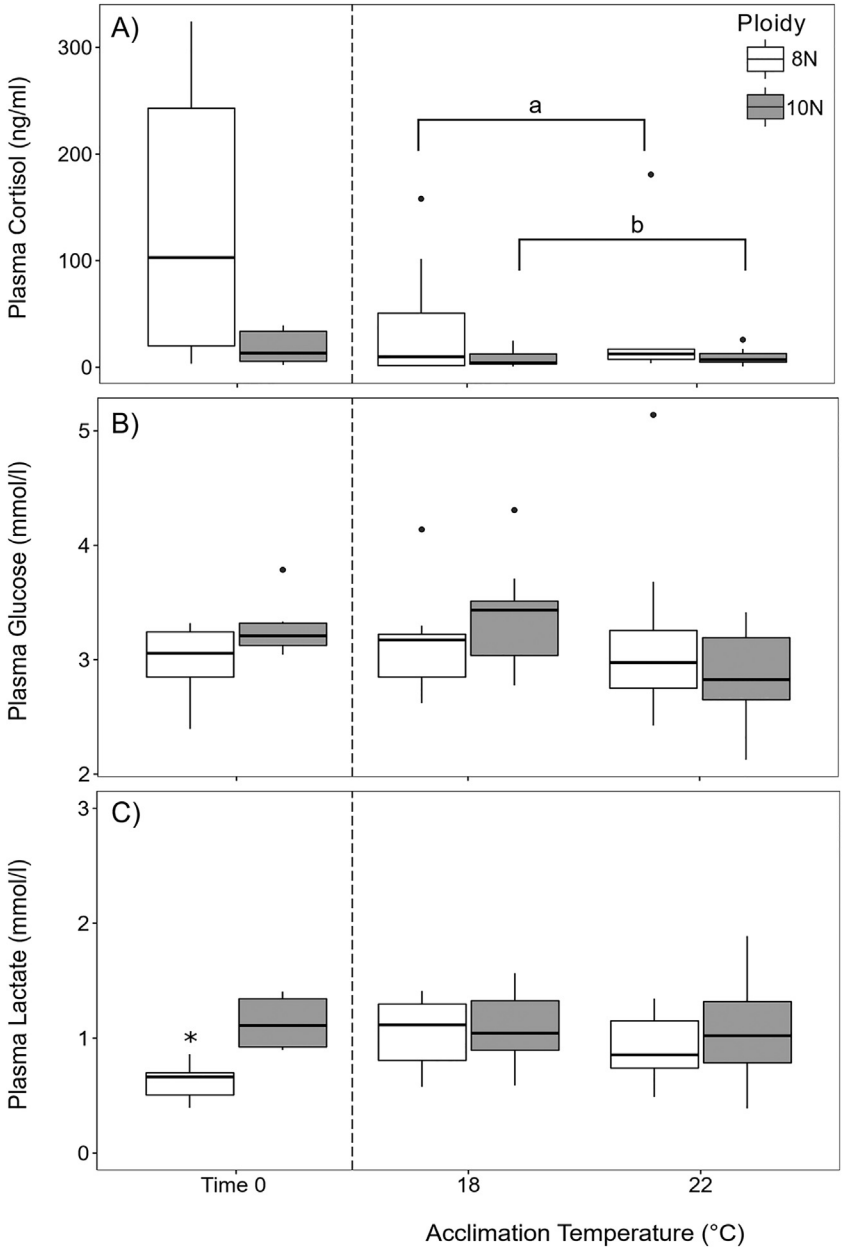

Fig. 1. Plasma cortisol (A), glucose (B) and lactate levels (C) of 8 N and 10 N white sturgeon before (Time 0, n = 6) and after a 6-week temperature acclimation (n = 12). Different letters denote significant differences between temperature or ploidies after temperature acclimation ($p < 0.05$). An asterisk (*) denotes significant differences between ploidies at Time 0 ($p < 0.05$).

and 15.5% of the variability in the data, respectively. The first two PCs were able to discriminate between ploidy (i.e. less overlap in groups; Fig. 4A) and were less able to discriminate between temperature acclimation treatments (Fig. 4B). RBC, Hct, and fork length were best at differentiating between ploidy, as might be expected. Plasma stress indices (cortisol, glucose and lactate) were negatively correlated with the plasma immune parameter measured (lysozyme). The highest contributing variable to the first two PCs is total erythrocyte counts. If all variables were contributing equally the expected contribution would be 5.56%. Total erythrocyte counts, hematocrit, mean erythrocyte volume, hemoglobin, mean erythrocyte hemoglobin, plasma lactate, total leukocyte counts, plasma cortisol, LDH Q_{10}, and fork length all have contributions > 5.56% and can therefore be considered important contributing factors in the first two PCs (Fig. 4C).

4. Discussion

Most research aimed at determining physiological differences between fish of different ploidies has been performed on salmonids (Benfey and Biron, 2000; Hyndman et al., 2003; Salimian et al., 2016), with only a single study in shortnose sturgeon (Beyea et al., 2005). White sturgeon are unique compared to salmonids in that triploids (12 N) of both sexes are fertile, and when they mate with diploids (8 N), they produce offspring with a ploidy level intermediate to that of their parents (10 N) (Drauch Schreier et al., 2011). To date little is known about diploid and triploid sturgeon, and even less is known about intermediate ploidies in fishes in general. Given the documented differences in physiology between salmonid fish of different ploidies, we were interested in examining if 8 N (diploid) and 10 N (intermediate

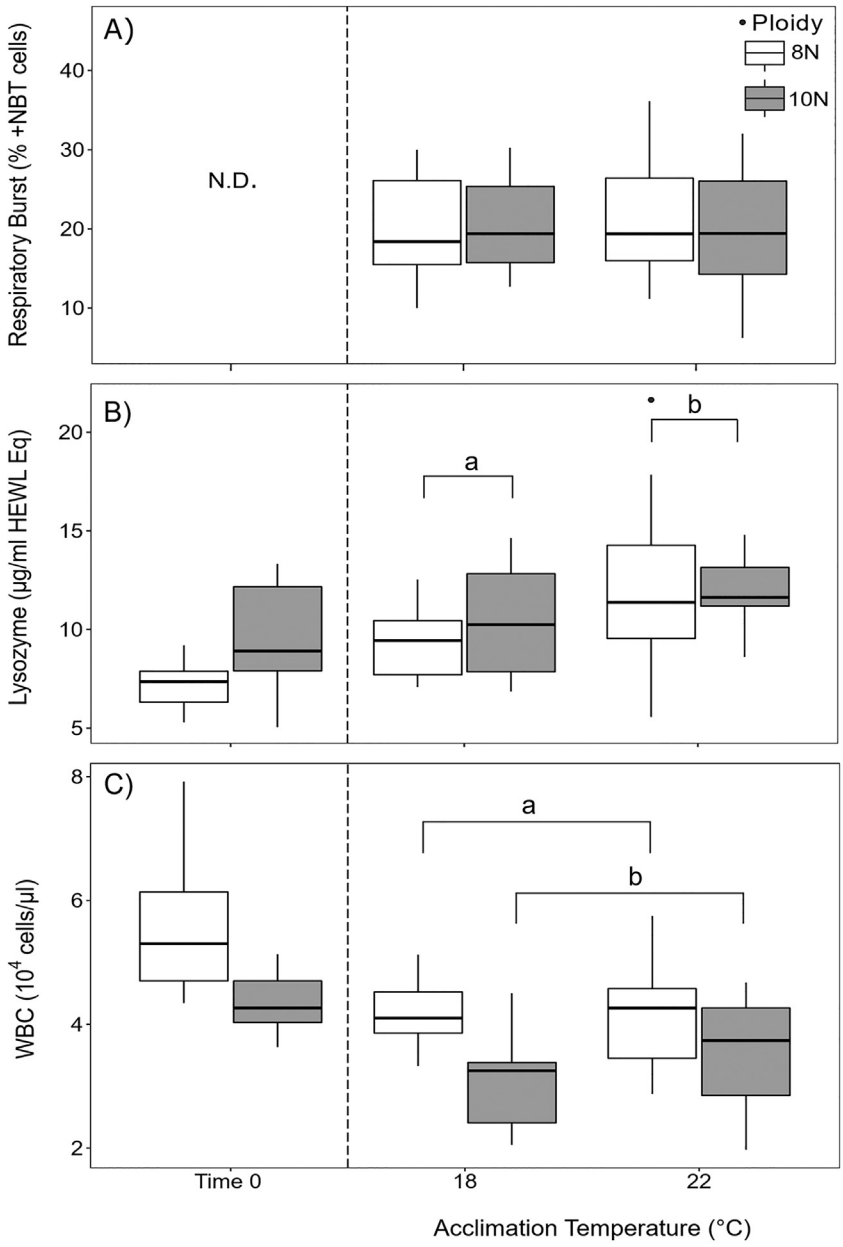

Fig. 2. Respiratory burst activities (A), plasma lysozyme (B) and total leukocyte counts (C) of 8 N and 10 N white sturgeon before (Time 0, n = 6) and after a 6-week temperature acclimation (n = 12). Different letters denote significant differences between temperature or ploidies after temperature acclimation ($p < 0.05$). An asterisk (*) denotes significant differences between ploidies at Time 0 ($p < 0.05$). N.D. = not determined, NBT = nitro blue tetrazolium.

ploidy) white sturgeon differed in their constitutive indices of stress, immunity, and metabolism under ambient and elevated temperature conditions. Our main results suggest that, although 10 N white sturgeon have larger but fewer blood cells, hematological and immunological responses to warm acclimation were not affected by ploidy (i.e. they exhibited the same magnitude of change in response to warmer water temperatures). Unexpectedly, 8 N sturgeon had more variable levels of plasma cortisol prior to and after acclimation at 18 °C compared to 10 N sturgeon (Fig. 1). The greatest physiological differences between 8 N and 10 N sturgeon were found in metabolic enzyme activities with 10 N fish having higher lactate dehydrogenase (LDH) activity under both acclimation temperatures. Additionally, 10 N sturgeon did not adjust citrate synthase (CS) activity in response to acclimation to elevated temperature, while 8 N sturgeon decreased CS activity in response to warming (Fig. 3; Table 2).

4.1. Constitutive indices of stress

To accommodate a larger genome, polyploids have larger but fewer cells, which results in a concurrent reduction in surface area to volume ratio at the cellular and tissue levels (Benfey, 1999). Differences in red blood cell counts (RBC) between ploidies have been found in diploid and triploid shortnose sturgeon (Beyea et al., 2005) as well as counts documented in several salmonid species (see Benfey, 1999 for a review). Our findings demonstrate that intermediate ploidies are similar to triploids in regards to having fewer red blood cell counts compared to diploids. Prior to (Time 0) and after acclimation, 10 N white sturgeon consistently had fewer erythrocytes compared to 8 N sturgeon, regardless of acclimation temperature (Table 2). Despite differences in cell size and number, the oxygen carrying capacity of 8 N and 10 N white sturgeon does not appear to differ. Compared to 8 N sturgeon,

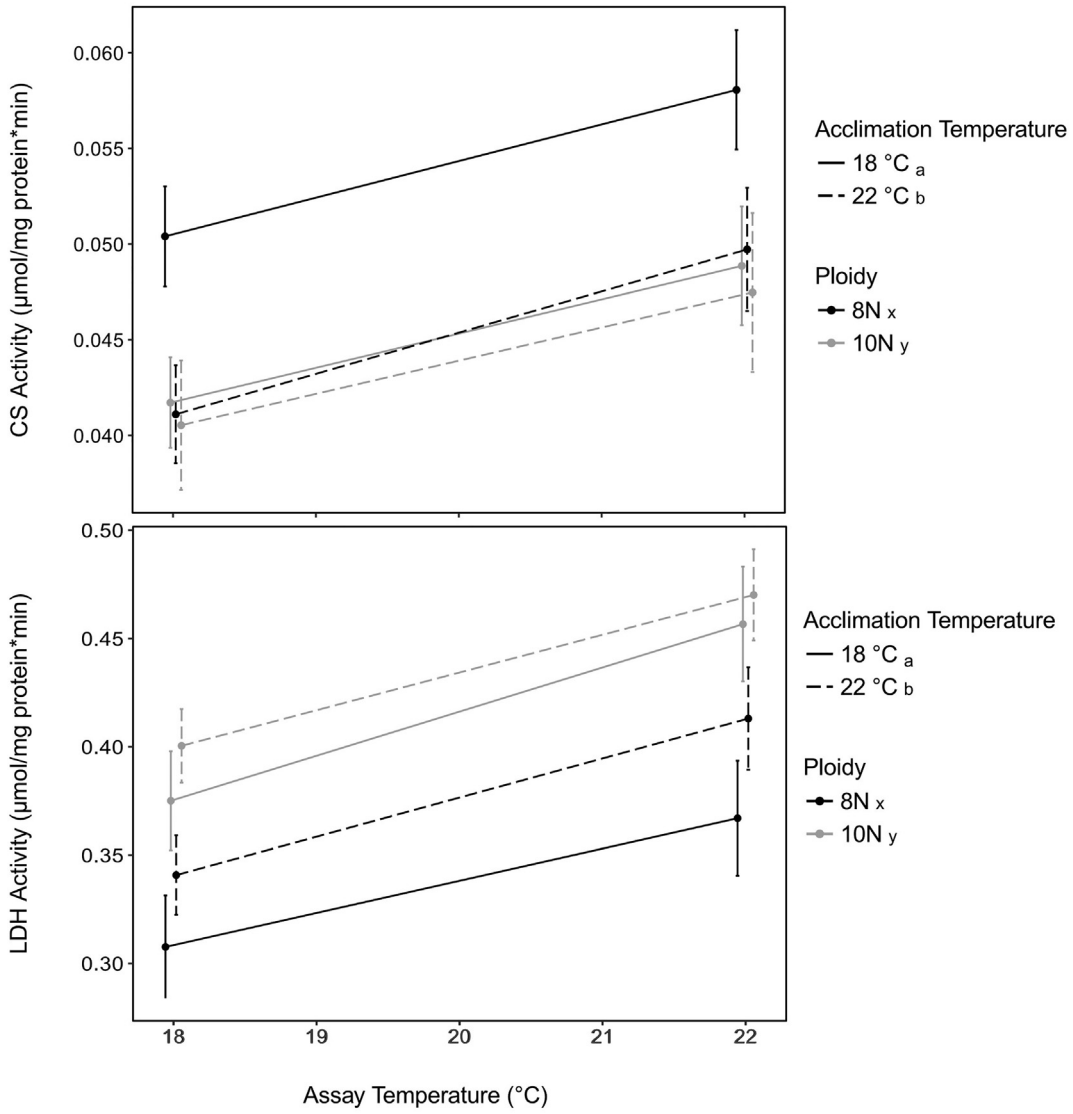

Fig. 3. Citrate synthase (A) and lactate dehydrogenase (B) activities of 8 N and 10 N white sturgeon after a 6-week temperature acclimation (n = 12). Different letters denote significant differences between acclimation temperature (a, b) and between ploidies (x, y) ($p < 0.05$).

Table 3
Enzyme temperature quotients (Q_{10}) of metabolic enzymes and enzyme ratios of 8 N and 10 N white sturgeon before and after a 6-week temperature acclimation (n = 12). Different letters denote significant differences between temperature and ploidies after temperature acclimation ($p < 0.05$). A dagger (†) denotes a significant difference between ploidies after temperature acclimation ($p < 0.05$). A plus sign (+) denotes a significant difference between acclimation temperatures ($p < 0.05$). LDH = lactate dehydrogenase, CS = citrate synthase.

	8 N		10 N	
	18 °C Acclimated	22 °C Acclimated	18 °C Acclimated	22 °C Acclimated
CS Q_{10}	1.43 ± 0.14[a]	1.61 ± 0.16[b]	1.48 ± 0.18[a,b]	1.48 ± 0.15[a,b]
LDH Q_{10}	1.58 ± 0.16	1.61 ± 0.19	1.66 ± 0.24	1.50 ± 0.16
LDH:CS[†,+]	11.1 ± 3.1	14.7 ± 2.7	16.3 ± 3.2	18.9 ± 7.2

10 N sturgeon had lower resting hematocrit (Hct), but had higher mean erythrocyte volume (MEV) and mean erythrocyte hemoglobin (MEH), consistently across acclimation temperatures (Table 2). Our results indicate that the hematological differences found in diploid and triploid sturgeon also hold true for comparisons made between diploid and intermediate ploidies of white sturgeon. Triploid shortnose sturgeon had lower resting Hct and higher MEV and MEH compared to diploid shortnose sturgeon (Beyea et al., 2005). Acclimation to 22 °C increased RBC, Hct, and Hb; however the magnitude of increase was comparable between ploidies (Table 2). Despite these differences in hematology, our results also demonstrate no effect of ploidy on Hb or mean erythrocyte hemoglobin concentration (MEHC), regardless of acclimation temperature. The lack of difference in Hb and MEHC between ploidies along with a higher MEH in 10 N fish, demonstrates that 10 N sturgeon have more hemoglobin per cell, likely to compensate for having fewer erythrocytes than 8 N sturgeon. Additionally, 10 N sturgeon exhibited higher MEV indicating a larger erythrocyte, which could accommodate more hemoglobin per cell compared to 8 N sturgeon. Given that 10 N sturgeon have two additional copies of their genome, further research could investigate if larger amounts of hemoglobin in erythrocytes are the result of increased gene expression from additional hemoglobin gene copies.

In several of the measured hematological parameters, large differences were observed between pre-acclimation (Time 0) and post-acclimation levels of 18 °C acclimated fish. Specifically, RBC, MEV, and MEH were notably different between Time 0 and post-acclimation fish held at 18 °C; this trend is consistent between both ploidies (Table 2). At

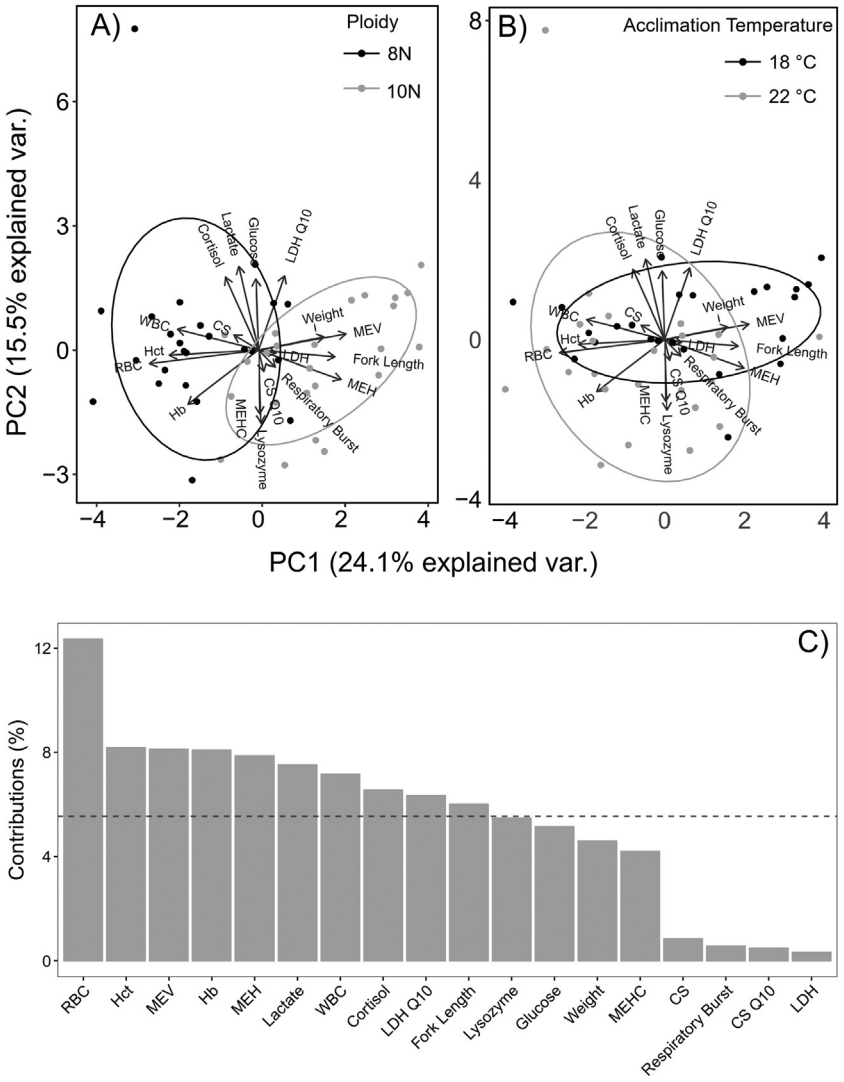

Fig. 4. Principal component analysis (PCA) biplot of the first 2 principal components grouped by ploidy (A) and grouped by acclimation temperature (B). A contributions plot (C). Height of the bars represent the percentage each variable contributes to the first 2 principal components. Horizontal dashed line represents the expected average contribution if all variable contributed equally. PC = principal component, RBC = total erythrocyte counts, Hct = hematocrit, Hb = hemoglobin, MEV = mean erythrocyte volume, MEH = mean erythrocyte hemoglobin, MEHC = mean erythrocyte hemoglobin concentration, WBC = total leukocyte counts, LDH = lactate dehydrogenase activity, CS = citrate synthase activity, Q_{10} = temperature quotient.

Time 0, tanks did not contain mixed ploidy groups, which may have impacted stress and hematological parameters if differences in behavior exist between ploidies. It is also worth noting that we unfortunately did not have replicate tanks for Time 0 data, meaning the differences we see at this point may be related to tank effect rather than ploidy. Additionally, MEV and MEH showed no significant difference between ploidies at Time 0, yet were significantly different between ploidies after acclimation (Table 2). A likely explanation for this disparity in results is due to sample size (n = 6 for Time 0 and n = 12 after acclimation); the authors recognize this as a limitation in our study.

Other studies have found no difference in blood oxygen binding affinity in diploid and triploid Atlantic salmon (Sadler et al., 2000b) nor in oxygen and carbon dioxide transport during exercise in diploid and triploid Chinook salmon (Bernier et al., 2004). These studies, along with our findings, indicate that ploidy does not appear to impact oxygen carrying capacity in fishes. In contrast to our predictions based on previous work comparing diploid and triploid fishes (Benfey, 1999), after acclimation, the intermediate ploidy (10 N) was not similar in constitutive bioindices of stress compared to diploid (8 N) white sturgeon. Specifically, 10 N white sturgeon had significantly lower plasma cortisol concentrations than 8 N white sturgeon regardless of acclimation temperature (Fig. 1A). Previous studies examining constitutive and induced cortisol levels of fishes of different ploidies have shown that diploid and triploid shortnose sturgeon (Beyea et al., 2005) and salmonids (Biron and Benfey, 1994; Benfey and Biron, 2000; Sadler et al., 2000a) have similar resting cortisol concentrations and respond similarly to an acute stressor. However, a recent study showed that resting cortisol levels were significantly higher in triploid rainbow trout fry, when compared to diploid fry (Salimian et al., 2016). Although the cortisol concentrations were significantly different in our study, it should be noted that the mean plasma cortisol concentrations of both ploidies after acclimation were comparable to resting plasma cortisol levels previously recorded in white sturgeon (Belanger et al., 2001) and can be considered “unstressed”. Additional research is needed to determine if 10 N and 8 N sturgeon would differ in the cortisol response to an acute stressor.

What was particularly noteworthy was the difference in variability in cortisol concentrations seen between ploidies. There was a large amount of variability in cortisol levels in 8 N sturgeon, especially before acclimation (each ploidy held in a separate tank) and this variability was significantly higher than the variability measured in 10 N sturgeon (Fig. 1A). Following acclimation to 18 °C and 22 °C (ploidies were intermixed in each tank), the variance within 8 N sturgeon was still significantly higher compared to the variance within 10 N sturgeon but was lower than the variability measured in 8 N sturgeon before acclimation. This phenomenon may be due simply to random sampling; however, pre-acclimation plasma cortisol concentrations were sampled and measured with an additional 12 fish (data not shown), with similar results and variability in the data. Furthermore, all samples were taken within 6 min of removing sturgeon from the tanks and there was no

measured increase in fish sampled later in the 6-min sampling window, providing evidence that the variability is likely not due to sampling. The high variability exhibited in 8 N sturgeon cortisol levels at Time 0 may also be due to a tank effect, as ploidies were separate by tank; however, high variability was also found in 8 N fish after temperature acclimation when ploidies were intermixed in tanks.

While additional research is needed to determine the cause of high cortisol variability, one possibility may be linked to social hierarchy. Numerous studies have assessed the relationship between constitutive cortisol levels and social hierarchy in multiple fish species and have demonstrated lower cortisol levels in dominant fish (Ejike and Schreck, 1980; Fox et al., 1997; Sloman et al., 2001; Doyon et al., 2003). More research is needed to determine if white sturgeon exhibit a social hierarchy and, if so, whether social ranking correlates to constitutive cortisol levels and if social ranking is impacted by ploidy. Regardless of the cause for variability in cortisol, the implication for farms and hatcheries is that some individual fish with chronically higher plasma cortisol levels may be more susceptible to stressors, which can result in slower growth and a higher incidence of disease (Barton et al., 1987; Pickering and Pottinger, 1989).

Although differences were found in resting cortisol level variability between 8 N and 10 N sturgeon, plasma glucose concentrations of white sturgeon of different ploidies did not differ. Plasma glucose concentrations before and after temperature acclimation were similar between ploidies and between acclimation temperatures (Fig. 1B). The plasma glucose concentrations measured in the current study are consistent with other reported resting values in white sturgeon (Crocker and Cech Jr., 1997), providing further evidence that after the temperature acclimation period sturgeon could be considered “unstressed”. Of note, plasma lactate values did differ between ploidies before temperature acclimation. Lower plasma lactate levels in 8 N sturgeon may indicate lower anaerobic activity or that 8 N sturgeon were utilizing lactate as a substrate in gluconeogenesis. Following the acclimation period, differences between 10 N and 8 N sturgeon plasma lactate levels no longer existed (Fig. 1C). Like plasma cortisol and glucose levels, plasma lactate levels were similar to resting levels previously documented in white sturgeon (Crocker and Cech Jr., 1997) and indicates that the fish were “unstressed” at the time of sampling.

Principal component analysis (PCA) was more capable of distinguishing between ploidies than temperature acclimation groups, supporting the conclusion that, although 8 N and 10 N sturgeon have inherent physiological differences, their responses to chronically elevated temperature are comparable, which may indicate that the temperatures used in this study were not in fact suboptimal. Further investigations into temperatures above what were used in this study may yield more insight into response of sturgeon of different ploidies to suboptimal temperatures. The PCA also revealed important response variables that explained the variability between 10 N and 8 N white sturgeon, including RBC, Hct, MEV, Hb, and MEH, emphasizing the importance of measuring hematological parameters when evaluating differences between fish of different ploidies (Fig. 4).

4.2. Constitutive indices of immunity

Constitutive immune capabilities as well as changes in immune parameters in response to warming were comparable between 8 N and 10 N white sturgeon. Ploidies did not differ in respiratory burst (RB) activity or plasma lysozyme activity. Similar to RBC, total leukocyte counts (WBC) differed between 8 N and 10 N sturgeon. As expected, 10 N sturgeon had lower WBC compared to 8 N sturgeon. This relationship was only significant after acclimation, but the lack of significance pre-acclimation was likely due to a small sample size (n = 6 before acclimation; Fig. 2). Based on our respiratory burst and plasma lysozyme analyses, phagocytosis and secretion of lysozyme are not impeded by the differences in cell size and subsequent cell surface area to volume ratios.

Of the three immune parameters assessed in this study, only plasma lysozyme was influenced by acclimation temperature. Higher plasma lysozyme activity was found in sturgeon acclimated to 22 °C compared to those acclimated to 18 °C (Fig. 2B). Previous studies have shown a similar positive relationship between plasma lysozyme activity and acclimation temperature in Atlantic halibut (Langston et al., 2002), Nile tilapia (Dominguez et al., 2005), and Mozambique tilapia (Ndong et al., 2007). In rainbow trout, multiple lysozymes can be expressed at the same time and different lysozymes have demonstrated differences in activity at elevated temperatures (Grinde et al., 1988). The higher lysozyme activity seen in the 22 °C acclimated fish may be due to the presence of a more efficient isoform of lysozyme or the increased production of more lysozyme. A lack of change in RBC and WBC between temperature acclimation groups in our study may be the result of an ability to adjust immune responses to environmental temperature, with the capacity to adjust being similar in 8 N and 10 N white sturgeon after a 6-week acclimation.

4.3. Metabolic enzyme activity

To date there have been limited studies to characterize whether fish of different ploidies are similar in their cellular metabolic capacity. Since having more DNA copies alters cell number and SA/V ratios, the number of mitochondria per cell and cellular oxygen transport could be influenced by ploidy and thereby alter cellular metabolic capacity. To our knowledge, only two studies have previously examined metabolic enzyme activities in fish of different ploidies. Lemieux et al. (2003) examined metabolic enzymes in diploid and triploid Arctic charr; however, data from fish of different ploidies were pooled to compare to another strain and no statistical analyses comparing diploid and triploid fish were reported. In a different study, female triploid catfish demonstrated higher liver glutamate pyruvate transaminase activity but lower liver cytosolic NADP-malate dehydrogenase and mitochondrial NAD-malate dehydrogenase activity; yet these differences may have been the result of lower circulating estradiol in the sterile triploid females (Biswas et al., 2006). In our study, white sturgeon of different ploidies differed in their activity of two metabolic enzymes. Specifically, 10 N white sturgeon had lower CS activity and higher LDH activity when compared to 8 N sturgeon. Additionally, warm acclimation increased LDH activity in both ploidies but decreased CS activity to a greater degree in 8 N sturgeon compared to 10 N sturgeon (Fig. 3). All the fish used in this experiment were sexually immature juveniles, meaning sterility and sex hormones did not play a role in the differences seen between ploidies.

Our results provide evidence that cellular metabolic capacity could differ between 8 N and 10 N white sturgeon at both ambient and elevated water temperatures. One explanation for the difference in CS activity between ploidies could be related to differences in cell number. CS activity can be used as a biomarker for mitochondrial volume, and therefore content (Larsen et al., 2012). If the number of mitochondria within cells remains constant between ploidies, the total number of mitochondria within a tissue would be reduced in 10 N sturgeon due to having fewer cells and would result in lower CS activity. The lower CS activity in 10 N sturgeon could also be due a lack of compensation of mitochondrial density within branchial cells, potentially resulting in a lower cellular aerobic capacity. Differences in surface area to volume ratios at the cellular level may also play a role in the dissimilarities in CS activity between ploidies. Having fewer but larger cells results in a lower SA/V ratio at the cellular and tissue level, which may impair oxygen transport to tissues and cells in fish with more genetic content (Benfey, 1999). Consequently, 10 N sturgeon may decrease the concentration of CS enzymes to match a more limited oxygen supply and would, therefore, need to compensate for this lower aerobic capacity. One mechanism to cope with reduced aerobic capacity is to utilize specific anaerobic pathways, such as conversion of pyruvate to lactate via LDH (Chandel, 2015). Higher LDH activity seen in 10 N sturgeon

compared to 8 N sturgeon after the six-week acclimation period to 18 °C as well as the significantly higher plasma lactate concentrations found in 10 N sturgeon compared to 8 N sturgeon prior to acclimation may support the idea that 10 N have a reduced aerobic capacity compared to 8 N sturgeon (Figs. 1C & 3B).

Warm acclimation to 22 °C resulted in distinct changes in branchial CS activity between 8 N and 10 N white sturgeon. After warm acclimation, CS activity was significantly lower in 8 N sturgeon acclimated to 22 °C compared to those acclimated to 18 °C, while 10 N sturgeon showed almost no change in CS activity in response to warm acclimation (Fig. 3A). Enzymes involved in oxidative energy production commonly undergo metabolic reorganization and show decreased activity after warm acclimation. Metabolic reorganization in response to warming has been demonstrated in multiple fishes, including green sunfish (Shaklee et al., 1977), Nile tilapia (Mwangangi and Mutungi, 1994), and threespine stickleback (Guderley et al., 2001). The lack of change in CS activity after warm acclimation in 10 N sturgeon suggests that they do not require or are unable to undergo metabolic reorganization with warming. Higher mortalities have been reported in triploids exercised at higher water temperatures (Hyndman et al., 2003), providing evidence of aerobic limitations in polyploids. Therefore, 8 N sturgeon may reduce CS activity at warmer temperatures because they can meet metabolic demand with fewer enzymes working at a faster rate, while 10 N sturgeon may already be at a reduced aerobic capacity due to a reduced SA/V ratio causing limitations on oxygen transport and, thus, cannot afford to reduce enzyme production at higher temperatures.

4.4. Conclusions

Overall, our data support the conclusion that 8 N and 10 N white sturgeon are similarly capable of acclimating their constitutive stress and innate immune parameters to warmer water temperatures. However, we did document that 8 N sturgeon differ in the variability in plasma cortisol levels, the cause of which is still unclear but may leave some individuals more susceptible to acute stressors that commonly occur on farms, such as handling and high stocking densities. What remains to be assessed is if white sturgeon of different ploidies have similar inducible stress and immune responses to acute stress and disease challenges, respectively. The difference in cellular metabolic enzyme activities of 8 N and 10 N sturgeon suggest that the aerobic capacity of 10 N sturgeon may be impaired at higher water temperatures, which is increasingly important in the context of global climate change. A difference in specific enzyme activity may translate more broadly to differences in whole organism aerobic performance at higher temperatures, which could have implications, such as higher mortalities in polyploid fish, for hatcheries and farms that stock white sturgeon of different ploidies. Although constitutive stress and immune parameters appear to be comparable between 8 N and 10 N white sturgeon, dissimilarities in variability of plasma cortisol and metabolic enzyme activity suggest key physiological differences between ploidies that could have implications for the health of white sturgeon at various facilities. Additionally, variability in growth rates between ploidies suggest there is much room for investigating the physiology of fish of different ploidies and emphasizes the importance of screening and managing ploidy on white sturgeon farms and hatcheries.

Acknowledgements

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