Journal of Thermal Biology 119 (2024) 103807

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Resetting thermal limits: 10-year-old white sturgeon display pronounced but reversible thermal plasticity

Theresa A. Weber [a], Angelina M. Dichiera [a,b,*], Colin J. Brauner [a]

[a] Department of Zoology, The University of British Columbia, Vancouver, British Columbia, Canada
[b] Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, USA

ARTICLE INFO

Keywords:
Phenotypic plasticity
CT_{max}
Climate change
Temperature

ABSTRACT

While many ectotherms improve thermal tolerance in response to prolonged thermal stress, little is known about the lasting effects of warm acclimation after returning to cooler temperatures. Furthermore, thermal stress may disproportionately impact threatened and endangered species. To address this, we repeatedly measured critical thermal maxima (CT_{max}; °C) and associated stress responses (hematocrit, hemoglobin concentration, plasma cortisol) of endangered subadult white sturgeon (*Acipenser transmontanus*) in response to control temperature (pre-acclimation; 14°C), after 1 month at either control or warm temperature (acclimation; 14°C or 20°C), and after one smonth following return to control temperature (post-acclimation; 14°C). While control fish demonstrated fairly repeatable thermal tolerance (interclass correlation coefficient = 0.479), warm-acclimated fish experienced a ~3.1°C increase in thermal tolerance and when re-acclimated to control temperature, decreased thermal tolerance ~1.9°C. Hematocrit, hemoglobin concentration, and final splenic somatic index (spleen mass relative to whole body mass, collected after post-acclimation CT_{max}) were not significantly different between control and treatment fish, suggesting no effects of warm acclimation on aerobic capacity. Plasma cortisol was significantly higher in control fish after pre-acclimation and post-acclimation CT_{max} trials, but importantly, acclimation temperature did not affect this response. Strikingly, final hepatosomatic index (relative liver size) was 45% lower in treatment fish, indicating warm acclimation may have lasting effects on energy usage and metabolism, even after reacclimating to control temperature. To our knowledge, these 10-year-old subadult sturgeon are the oldest sturgeon experimentally tested with regards to thermal plasticity and demonstrate incredible capacity for thermal acclimation relative to other fishes. However, more research is needed to determine whether the ability to acclimate to warm temperature may come with a persistent cost.

1. Introduction

In the past decade, anthropogenic activities have increased global surface temperatures +1.1°C above pre-industrial levels (IPCC, 2023). Even small rises in global temperatures exponentially increase extreme weather events, causing more frequent, intense heatwaves (Meehl and Tebaldi, 2004; Oliver et al., 2018; IPCC, 2023). Ectothermic animals like fishes are particularly affected by changes in environmental temperature as it governs their fitness, performance, and distribution (Beitinger et al., 2000; Fry, 1947; Jutfelt et al., 2021; Perry et al., 2005; Sunday et al., 2019). With steadily rising global temperatures and increased thermal variability, there is a push to understand and predict species' thermal vulnerability.

Often, measures of acute warming tolerance (i.e., critical thermal maxima, CT_{max}) are used to characterize thermal vulnerability and provide useful information for predicting responses to warming temperatures (Deutsch et al., 2008; Evans et al., 2015; Sunday et al., 2011, 2012, 2019). Many fishes have some capacity for acclimation, wherein they can adjust their physiology to the surrounding environmental conditions and increase the probability of survival (Beaman et al., 2016; Morley et al., 2019; Seebacher et al., 2015). How *much* an individual can increase their upper thermal limit varies between species ecology, evolutionary history, and life stage (Pottier et al., 2022; Dahlke et al., 2020). And though the plasticity of upper thermal limits may be limited by a physiological ceiling (Araújo et al., 2013; Bennett et al., 2021; Morgan et al., 2020; Sandblom et al., 2016), it could help fishes cope with warming – at least in the short-term.

White sturgeon (*Acipenser transmontanus*) are the largest and one of

---

* Corresponding author.
E-mail address: dichiera@vims.edu (A.M. Dichiera).

https://doi.org/10.1016/j.jtherbio.2024.103807
Received 5 September 2023; Received in revised form 22 January 2024; Accepted 23 January 2024

the longest living freshwater fish in North America (COSEWIC, 2012). Valued for their roe, these late-maturing fish were nearly decimated by overharvesting in the early 1900s, and many populations remain threatened and endangered today (COSEWIC, 2012; Hildebrand et al., 2016). With issues of global climate change, increasing attention is being paid to how these fish respond to thermal stress, and if they display phenotypic plasticity with warm temperatures (Earhart et al., 2023; Han et al., 2012; Lee et al., 2016; Penman et al., 2023; Rodgers et al., 2019). Like many ectothermic species, studies have demonstrated that warm acclimation increases CT_{max} in sturgeon species, such as juvenile shortnose sturgeon (*Acipenser brevirostrum*; Bard and Kieffer, 2019; Kieffer and Bard, 2022; Zhang and Kieffer, 2014; Ziegeweid et al., 2008), juvenile lake sturgeon (*Acipenser fulvescens*; Bugg et al., 2020; Bugg et al., 2023), and young-of-the-year white sturgeon (Penman et al., 2023). Early life stages of white sturgeon also demonstrate exceptional thermal plasticity, increasing upper thermal limits by ~8.5°C when acclimated to treatments 7°C warmer than ambient temperature (Earhart et al., 2023).

Yet, little is known about the acclimation capacity of ectotherms in later life stages, or if they experience persistent effects *after* chronic thermal exposure ($\geq$ three weeks). A recent meta-analysis indicated that in general, early thermal environment does not have a persistent effect on thermal tolerance (Pottier et al., 2022). However, juvenile ectotherms acclimated to warm temperatures tended to retain some elevated heat tolerance after being re-acclimated to common garden conditions, in contrast to embryonic ectotherms that went through similar experimental design (Pottier et al., 2022). Fewer studies have targeted the sub-adult and adult life stage to investigate whether the effects of warm acclimation are reversible (i.e., maintaining a warm-acclimated phenotype is too costly) or if chronic thermal exposure has persistent effects after returning to control temperatures.

Stress is usually assessed by quantifying cortisol (stress hormone) production, and in striped bass (*Morone saxatilis*), plasma cortisol levels are significantly higher in warm-acclimated fish post-CT_{max} when compared with fish at control temperatures pre- or post-CT_{max}, demonstrating a combined effect of acute and chronic thermal stress (Penny and Pavey, 2021). Stress can also cause red blood cell swelling (Nikinmaa, 2011) and/or increased circulating red blood cells via splenic contraction (Pearson and Stevens, 1991) – changes to blood oxygen transport characteristics that may allow them to keep up with the elevated energetic demands caused by warming (Fry and Hart, 1948; Richter et al., 2010). Acute thermal stress increases hematocrit in some species of fish, like salmonids (Vargas-Chacoff et al., 2018; Muñoz et al., 2018; Gomez Isaza and Rodgers, 2022) and green sturgeon (*Acipenser medirostris*; Sardella et al., 2008), whereas in other species, acclimation to warm temperatures, and not acute thermal stress, increases hematocrit (Bard and Kieffer, 2019; Spear and Kieffer, 2016; Zhang and Kieffer, 2014). Furthermore, warm acclimation has been shown to decrease relative liver size (hepatosomatic index) in some fishes, which may aid in mobilizing energy stores like glycogen and lipids, a potential metabolic trade-off for increased growth at elevated temperatures (Sandblom et al., 2016; Bugg et al., 2020). However, to our knowledge, it is unknown if these responses to warm acclimation persist after re-acclimation to control temperatures.

Here we characterized the effects of prolonged thermal acclimation on 10-year-old subadult white sturgeon and the subsequent effects of returning to control temperature. We repeatedly measured CT_{max} to understand if sturgeon exhibit thermal plasticity. We also measured post-CT_{max} stress responses (hematocrit, hemoglobin concentration, and plasma cortisol concentration) to elucidate changes to their physiology during warm acclimation that may enable plasticity, and changes after returning to control temperature that may demonstrate persistent effects (see Fig. 1 for experimental design). CT_{max} and post-CT_{max} stress responses were initially measured for all fish at 14°C (pre-acclimation), measured after treatment fish underwent a one-month acclimation at 20°C (acclimation), and finally measured after treatment fish were returned to 14°C and re-acclimated for one month (post-acclimation). Fish were sampled for splenic somatic index and hepatosomatic index after post-acclimation CT_{max} to identify any persistent changes to circulating red blood cells or energetic stores, respectively, associated with warm acclimation. We hypothesized that subadult white sturgeon would demonstrate thermal plasticity with no persistent effects of warm

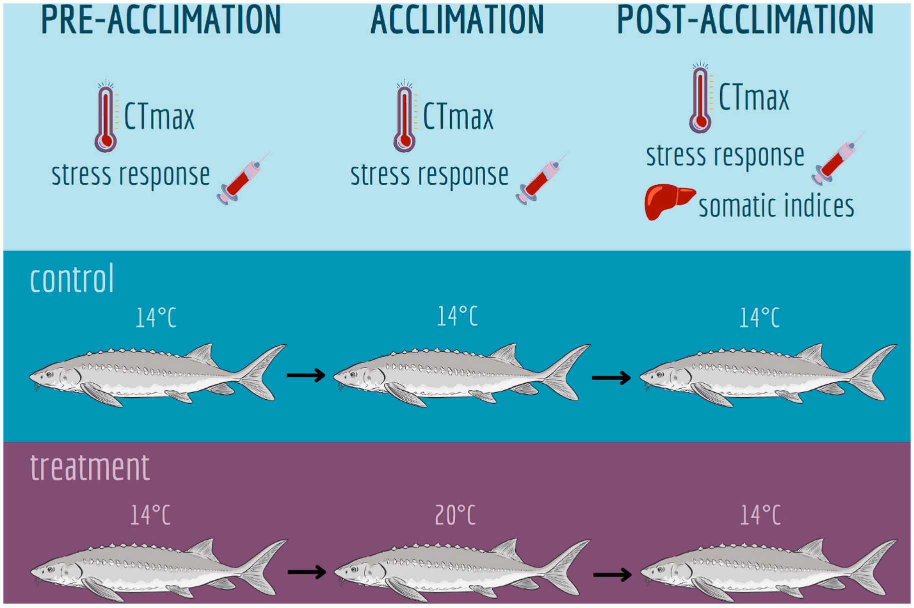

Fig. 1. Experimental design. Critical thermal maxima (CT_{max}) and post-CT_{max} stress responses (hematocrit, hemoglobin concentration, and plasma cortisol concentration) were measured for subadult white sturgeon maintained at 14°C (control) or sturgeon which underwent a warm acclimation at 20°C and were re-acclimated to 14°C (treatment). CT_{max} and post-CT_{max} stress responses were initially measured for all fish at 14°C (pre-acclimation), then measured after treatment fish underwent a one-month acclimation at 20°C (acclimation), and finally measured after treatment fish were returned to 14°C and re-acclimated for one month (post-acclimation). After the post-acclimation CT_{max}, control and treatment fish were sampled for somatic indices (splenic somatic index and hepatosomatic index). White sturgeon illustrations by Rush Dhillon.

acclimation. We predicted: (1) warm acclimation would increase CT$_{max}$; (2) re-acclimation to control temperature would result in a return to pre-acclimation CT$_{max}$; and (3) warm acclimation would alter post-CT$_{max}$ stress responses (e.g., increased hematocrit, hemoglobin, and/or plasma cortisol) but there would not be sustained changes upon re-acclimation to control temperature, indicative of thermal plasticity.

2. Materials and methods

2.1. Experimental animals

Young-of-the-year white sturgeon were originally obtained from Vancouver Island University (Nanaimo, British Columbia) in September 2013, and housed at the University of British Columbia (Vancouver, British Columbia). All fish were PIT tagged for individual identification and tracking throughout the study. Prior to the start of this study in August 2022, fish (n = 32) were held in a single recirculating aquaculture system consisting of two 5 m^3 tanks (n = 16 per tank) and maintained at 14.01 ± 0.02°C (a standard holding temperature) with a 12:12h light-dark cycle. Throughout the study, temperature and dissolved oxygen (DO; maintained >95%) were checked daily, ammonia (maintained <0.25 ppm) and pH (7.28 ± 0.03) were checked weekly, and water changes conducted periodically. Fish were fed approximately 1% body mass daily as a maintenance diet throughout the study, with exception for fasting 48h before testing. This study was conducted in accordance with the University of British Columbia Animal Care and Use Committee guidelines and approved under the permit A19-0284.

2.2. Experimental design

Sturgeon were randomly selected (n = 8 per tank, n = 16 total; initial mass ~3.1 kg; see Fig. S1 for experimental design details) and tested at standard holding temperature to establish pre-acclimation CT$_{max}$. Fish were tested in groups of four individuals, as these repeated measures trials relied on complete recovery and previous research indicates sturgeon recover from acute thermal stress better in groups (Yusishen et al., 2020). We measured CT$_{max}$ using a modified approach from Strowbridge et al. (2021). Briefly, a reservoir tank of water (~1050 l) was heated to ~60°C the day prior, and was used as the heated water source for each trial. At the start of each trial, fish were transferred to the experimental tank (~500 l) at their holding temperature and allowed to become accustomed to the tank for 1 h. Water was then pumped from the heated water source at a rate sufficient to result in a change in tank temperature of +0.3°C min^{-1} using small adjustments to a flow valve. Two circulating pumps maintained flow within the experimental tank to reduce any thermal stratification, and air stones were used to maintain aeration throughout the trial. During the trial, temperature was recorded each minute using two YSI 2030 Pro probes, which were placed on each end of the experimental tank to ensure the experimental tank was heating equally (±0.1°C). CT$_{max}$ was recorded as the temperature when loss of equilibrium (LOE) occurred. LOE was determined when the fish could no longer maintain upright orientation (i.e., rolling over and exposing their belly for >5 s). Immediately after LOE, fish were removed and placed in a light anesthetic bath (150 mg l^{-1} MS-222; 300 mg l^{-1} NaHCO$_3^-$). Fish were quickly measured for fork length (FL; measured to the nearest 0.5 cm) and whole-body mass (measured to the nearest 10 g) and 1 ml whole blood was drawn from the caudal vein using a heparinized needle and syringe. Fish were then recovered in an aerated recovery tank at 20°C (an intermediate temperature between their CT$_{max}$ and holding temperature) for 1 h, before returning to their original tanks for a few days before the subsequent treatment. After the pre-acclimation CT$_{max}$ trials, survival was 100%.

To assess the effect of acclimation temperature on CT$_{max}$ and stress responses, these fish (n = 16) were then identified by PIT tag, and randomly separated into either control (14°C) or treatment (20°C) groups for one month. Control fish were maintained in the original recirculating aquaculture system consisting of two 5 m^3 tanks (n = 4 per tank), and maintained at 14.01 ± 0.02°C (Tank A$_1$) and 14.00 ± 0.03°C (Tank B$_1$). Treatment fish were moved to a second recirculating aquaculture system consisting of two 5 m^3 tanks (n = 4 per tank), wherein temperature was increased +2°C per day for 3 days to reach warm temperatures. These treatment tanks were maintained at 19.48 ± 0.28°C (Tank A$_2$) and 19.25 ± 0.43°C (Tank B$_2$). Untested fish (n = 16) were equally split between the control and treatment recirculating aquaculture systems for a final tank density of 8 fish per tank. After a one-month acclimation period, previously tested fish underwent a second round of CT$_{max}$ trials as described above, where fish were tested at their respective holding temperatures and were again sampled post-CT$_{max}$ for blood, FL, and whole-body mass. Fish were then recovered in an aerated recovery tank at 20°C or 24°C for 1 h before returning to their respective tanks. After the acclimation CT$_{max}$ trials, two fish with stress injuries (one from each the control and treatment groups) were humanely euthanized as a preventative measure.

To assess the effect of re-acclimation to the control temperature after warm acclimation, the treatment group tanks were decreased −2°C per day until they reached 14°C, and then the treatment fish were split into the original recirculating aquaculture system consisting of two 5 m^3 tanks and maintained at 13.98 ± 0.03°C (Tank A$_1$) and 13.97 ± 0.04°C (Tank B$_1$). Fish were re-acclimated to 14°C for one month (n = 7 tested fish per tank, n = 8 untested fish per tank, n = 15 total fish per tank). A final round of CT$_{max}$ testing occurred post-acclimation at their holding temperature after which fish were euthanized (500 mg l^{-1} MS-222; 1 g l^{-1} NaHCO$_3^-$) and terminally sampled for blood, FL, whole-body mass, as well as spleen and liver mass (measured to the nearest 1 g).

To measure thermal plasticity with warm acclimation, we calculated the acclimation response ratio (ARR) for the treatment group (Bugg et al., 2020; Zhang and Kieffer, 2014), where average CT$_{max}$ at pre-acclimation temperature (14°C) was subtracted from average CT$_{max}$ at acclimation (20°C) and divided by the change of acclimation temperature (+6°C). To measure thermal plasticity with re-acclimation, we calculated the acclimation response ratio for the treatment group where average CT$_{max}$ at acclimation (20°C) was subtracted from average CT$_{max}$ at acclimation (14°C) and divided by the change of acclimation temperature (−6°C).

2.3. Stress response measurements and tissue indices

Whole blood sampled from all fish post-CT$_{max}$ at pre-acclimation (n = 8 per treatment), acclimation (n = 8 per treatment), and post-acclimation (n = 7 per treatment) were used to measure hematocrit, hemoglobin concentration, and plasma cortisol. Hematocrit (Hct; %) was measured in triplicate, where whole blood samples were collected into micro-capillary tubes, centrifuged for 2 min at 10,000 rpm, and the percentage of packed red blood cells volume to whole blood volume was calculated. The average of the triplicate Hct measures was taken for each individual at each time point. Hemoglobin concentration (Hb; mM) was measured using the standard Drabkin's method, diluting 4 μl of whole blood in 1 ml Drabkin's solution. Each individual sample was measured in triplicate at 540 nm, and average Hb was calculated using absorbance (A), accounting for 1:250 dilution factor, 4 Heme groups, and using an extinction coefficient of 11 mmol l^{-1} cm^{-1}:

$$Hb = \frac{250(A/11)}{4}$$

Mean Corpuscular Hemoglobin Concentration (MCHC) was then calculated by dividing Hb concentration by Hct:

$$MCHC = Hb \Bigg/ \left(\frac{Hct}{100}\right)$$

The remaining whole blood samples were centrifuged for 2 min at 10,000 rpm. Plasma and packed red blood cells were separated, and plasma was stored at −80°C until cortisol analyses. Splenic Somatic

Index (SSI) and Hepatosomatic Index (HSI) were calculated by dividing the wet spleen or liver mass by the wet whole-body mass and multiplying by 100. Fulton's condition factor (Fulton, 1904; Nash et al., 2006) was calculated as follows:

$$Condition\ factor = \frac{Whole\ body\ mass\ (g)\ x\ 100}{Fork\ length\ (cm)^3}$$

Plasma cortisol concentration was measured using a commercially available cortisol ELISA assay kit (Eagle Biosciences, kit C0R31-K01). Following the manufacturer's instructions, after color development, absorbance was measured at 450 nm using a 96-well plate spectrophotometer (SpectraMax 190, Molecular Devices, Softmax® Pro) and concentrations (ng ml^{-1}) were calculated using the standards provided, according to the manufacturer's guidelines. Each individual sample, control, and standard were run in duplicate per manufacturer's guidelines.

2.4. Statistical analyses

All statistical analyses were performed using RStudio (version 2022.12.0; http://www.R-project.org/) with a significance level (α) of 0.05. Prior to analyses, all data were tested for normality using Shapiro-Wilk's tests, and homogeneity of variance using Levene's tests. If normality was violated, outliers were identified (±2 SD) and removed from the dataset. HSI was analyzed using a Student's t-test, and SSI was analyzed using Welch's *t*-test for unequal variances, as homogeneity of variance was violated. For all other metrics, a series of two-way repeated measures ANOVAs were performed using the packages *afex* (Singmann et al., 2015) and *emmeans* (Lenth et al., 2018). For each metric, we tested for main effects of treatment (control or treatment) and CT_{max} trial (pre-acclimation, acclimation, or post-acclimation), and the interaction of treatment and trial. To account for the repeated measures design, we specified an error term that contained the subject (individual fish identified by their unique PIT tag codes) and the within-subject variable (trial). If treatment and trial had a significant interaction, Tukey HSD post-hoc tests were conducted, and results are reported with Tukey adjusted p-values. Detailed outputs of Tukey HSD post-hoc tests can be found in Table S1. In addition, to assess any tank effects, all statistical tests were run without a replicate tank (Tanks A_1, B_1, A_2, or B_2) for all three trials, and we found tank replicate did not affect the overall interpretation of our main results (see Table S2, Fig. S2, and Supplemental Text for details). To determine the repeatability of CT_{max}, Pearson's correlation coefficients were calculated for correlations between each trial, and package *rptR* (Stoffel et al., 2017) was used to calculate the repeatability across all trials (also known as the intraclass correlation coefficient). All data are reported as mean ± S.E.M. in Tables S3–5 and represented in Figs. 2–7.

3. Results

3.1. The effect of acclimation temperature on thermal tolerance

For CT_{max}, there was no main effect of treatment type ($F_{1,12} = 2.80$; $p = 0.120$), but a main effect of CT_{max} trials ($F_{1.64,19.74} = 17.07$; $p < 0.001$) and an interaction between treatment and trials ($F_{1.64,19.74} = 7.19$; $p = 0.007$). Following interactive effects, Tukey HSD post-hoc tests showed there were no significant differences between control and treatment CT_{max} at pre-acclimation ($t = 0.13$; $df = 12$; $p = 0.896$; Tables S1 and S3; Fig. 2). After warm acclimation at 20°C, the treatment group significantly increased their CT_{max} from their original pre-acclimation CT_{max} ($t = -6.81$; $df = 12$; $p < 0.001$) and had a significantly higher CT_{max} than the control group ($t = -3.51$; $df = 12$; $p = 0.004$). When re-acclimated to 14°C (post-acclimation), the treatment group significantly decreased their CT_{max} from acclimation CT_{max} at 20°C ($t = 4.81$; $df = 12$; $p = 0.001$) and was not significantly different from their pre-acclimation CT_{max} ($t = -2.60$; $df = 12$; $p = 0.056$) nor control post-acclimation CT_{max} ($t = -1.36$; $df = 12$; $p = 0.198$). Warm-acclimated sturgeon exhibited an acclimation response ratio of 0.52 after one month of acclimation at 20°C (i.e., 0.52°C increase in CT_{max} per 1°C of acclimation). Conversely, warm-acclimated sturgeon exhibited an acclimation response ratio of 0.31 after re-acclimating to 14°C for one month (i.e., 0.31°C decrease in CT_{max} per 1°C of acclimation).

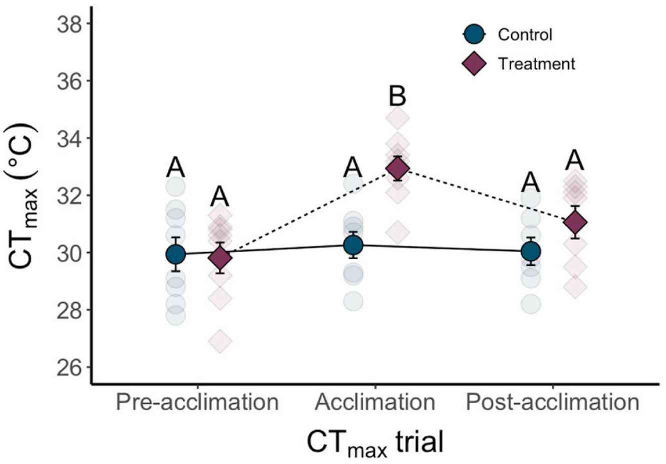

Fig. 2. CT_{max} values of subadult white sturgeon maintained at 14°C (control) or sturgeon which underwent a warm acclimation at 20°C and were re-acclimated to 14°C (treatment). CT_{max} were repeatedly measured for all fish three times: at 14°C (pre-acclimation), after treatment fish underwent a one-month acclimation at 20°C (acclimation), and after treatment fish were returned to 14°C and re-acclimated for one month (post-acclimation). There was a significant interaction between trial and treatment (two-way repeated measure ANOVA; $F_{1.64,19.74} = 7.19$; $p = 0.007$). Letters denote statistically significant differences within treatment as detected by subsequent Tukey HSD post-hoc analysis (see Results and Table S1 for details). Individual data points (n = 8 for each group at pre-acclimation and acclimation, and n = 7 for each group at post-acclimation) are overlayed with mean ± S.E.M. (control presented in blue, treatment presented in purple). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

There was no main effect of treatment on body mass ($F_{1,12} = 0.15$; $p = 0.708$), fork length ($F_{1,12} = 0.00$; $p = 0.966$), and Fulton's condition factor ($F_{1,12} = 0.48$; $p = 0.500$), nor was there a significant interaction between treatment and trial for body mass ($F_{1.37,16.42} = 2.58$; $p = 0.120$), fork length ($F_{1.47,17.62} = 0.87$; $p = 0.403$), and Fulton's condition factor ($F_{1.31,15.72} = 0.50$; $p = 0.538$). There was a main effect of trial on body mass ($F_{1.37,16.42} = 19.27$; $p < 0.001$) and Fulton's condition factor ($F_{1.31,15.72} = 7.54$; $p = 0.010$), but not fork length ($F_{1.47,17.62} = 2.67$; $p = 0.109$; Table S4; Fig. 3), suggesting that all fish improved their condition by gaining mass throughout the study.

3.2. Repeatability of CT_{max}

The repeatability of CT_{max} for control fish that performed all three CT_{max} trials (n = 7) was 0.479 (95% C.I.: 0.146–0.738; Fig. 4A). Pearson's correlation demonstrated that repeatability was highest between subsequent trials (CT_{max} trials 1 and 2: $R = 0.688$; CT_{max} trials 2 and 3: $R = 0.575$) but was reduced between CT_{max} trials 1 and 3 ($R = 0.268$; Fig. 4B).

3.3. The effect of acclimation temperature on stress responses

There were no significant differences in hematological responses between control and treatment fish across the three trials, nor an interaction between treatment and trials ($p > 0.05$), indicating warm acclimation had no significant effect on Hct, Hb concentration, and

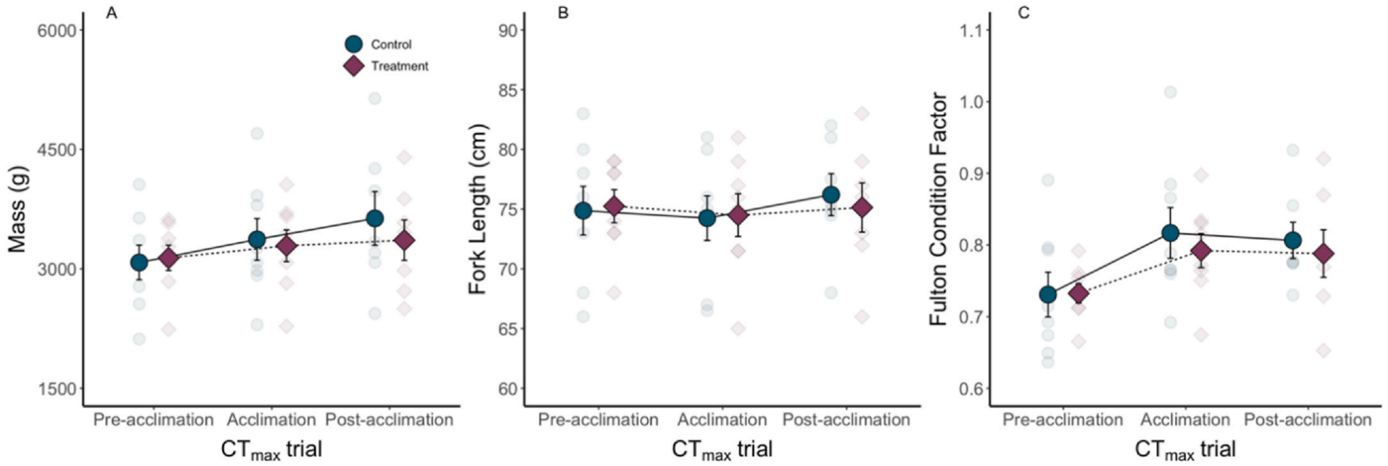

Fig. 3. Body condition of subadult white sturgeon maintained at 14°C (control) or sturgeon which underwent a warm acclimation at 20°C and were re-acclimated to 14°C (treatment) across repeated CT_{max} trials. There was no main effect of treatment on body mass (**A**), fork length (**B**), and Fulton's condition factor (**C**), nor was there a significant interaction between treatment and trial for body mass, fork length, and Fulton's condition factor (two-way repeated measures ANOVA; $p > 0.05$; see Results for details). There was a main effect of trial on body mass ($F_{1.37,16.42} = 19.27$; $p < 0.001$) and Fulton's condition factor ($F_{1.31,15.72} = 7.54$; $p = 0.010$) but not fork length ($F_{1.47,17.62} = 2.67$; $p = 0.109$), suggesting that all fish improved their condition by gaining mass throughout the study. Individual data points ($n = 8$ for each group at pre-acclimation and acclimation, and $n = 7$ for each group at post-acclimation) are overlayed with mean ± S.E.M. (control presented in blue, treatment presented in purple). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

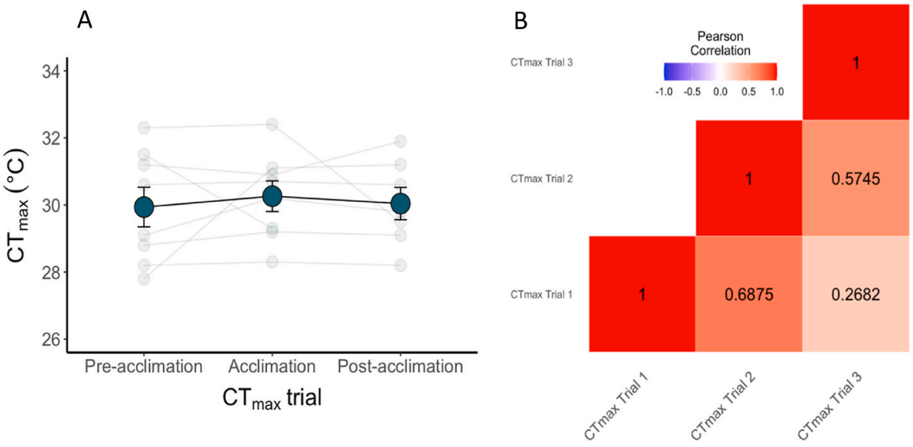

Fig. 4. Repeatability of CT_{max} trials of subadult white sturgeon maintained at 14°C (control). (**A**) CT_{max} values for control group across three trials. Individual data points and lines ($n = 8$ at pre-acclimation and acclimation, and $n = 7$ at post-acclimation) are overlayed with mean ± S.E.M. (**B**) Heatmap of the Pearson correlation coefficients between each CT_{max} trial. CT_{max} trials 1, 2, and 3 correspond with pre-acclimation, acclimation, and post-acclimation trials, respectively, but only for control fish here.

MCHC (Table S5; Fig. 5). There was no main effect of treatment type ($F_{1,11} = 0.73$; $p = 0.410$), no main effect of CT_{max} trials ($F_{1.78,19.55} = 3.31$; $p = 0.063$) nor an interaction between treatment and trials ($F_{1.78,19.55} = 0.14$; $p = 0.846$) on Hct. There was no main effect of treatment type ($F_{1,12} = 0.29$; $p = 0.600$), no main effect of CT_{max} trials ($F_{1.51,18.14} = 2.12$; $p = 0.157$) nor an interaction between treatment and trials ($F_{1.51,18.14} = 0.11$; $p = 0.846$) on Hb. There was no main effect of treatment type ($F_{1,10} = 0.83$; $p = 0.384$), no main effect of CT_{max} trials ($F_{1.44,14.44} = 0.88$; $p = 0.404$) nor an interaction between treatment and trials ($F_{1.44,14.44} = 0.04$; $p = 0.921$) on MCHC. There were likewise no main effects of treatment ($F_{1,11} = 3.77$; $p = 0.078$) or trial ($F_{1.88,20.72} = 0.61$; $p = 0.543$) on plasma cortisol concentrations; however, there was a significant interaction between treatment and trial ($F_{1.88,20.72} = 3.87$; $p = 0.039$; Table S5; Fig. 6). Following interactive effects, Tukey HSD post-hoc tests showed control fish had significantly higher plasma cortisol concentrations than treatment fish at pre-acclimation CT_{max} ($t = 3.21$; $df = 11$; $p = 0.008$) and post-acclimation CT_{max} ($t = 0.04$; $df = 11$; $p = 0.036$), but not acclimation CT_{max} ($t = 0.71$; $df = 11$; $p = 0.705$). There were no differences in plasma cortisol concentrations across CT_{max} trials for either control or treatment fish ($p > 0.05$; see Table S1 for details), suggesting control fish maintained higher plasma cortisol concentrations from the start of the study. Importantly, plasma cortisol concentrations measured at pre-acclimation were collected in the same manner and from fish randomly selected from the same tank conditions (14°C) suggesting differences in plasma cortisol concentrations between groups were due to random interindividual variation (i.e., not due to effects of warm acclimation). Splenic somatic index was not significantly different between control and treatment groups ($t = -0.51$; $df = 7.54$; $p = 0.628$). Treatment fish had a significantly lower hepatosomatic index than the control fish ($t = 5.23$; $df = 12$; $p < 0.001$; Fig. 7).

4. Discussion

For long-lived, slow-growing ectotherms like white sturgeon,

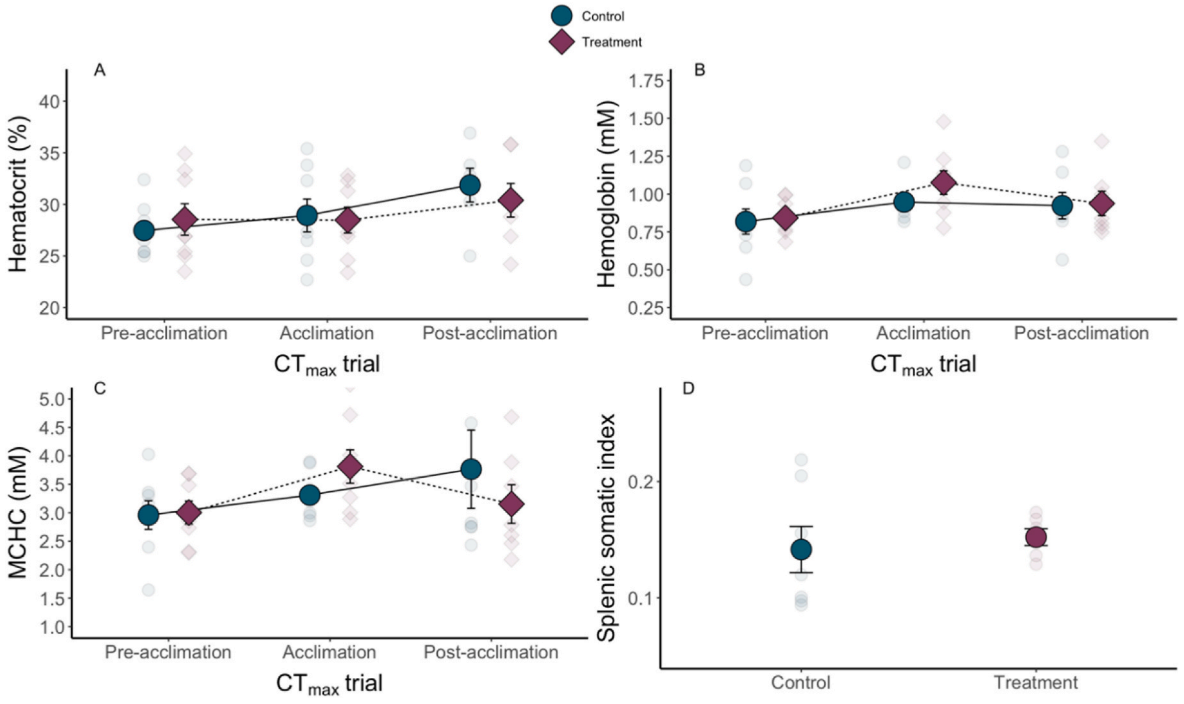

Fig. 5. Hematological response of subadult white sturgeon maintained at 14°C (control) or sturgeon which underwent a warm acclimation at 20°C and were re-acclimated to 14°C (treatment) immediately after CT_{max} trials. There was no main effect of treatment or trial, nor an interaction between treatment and trial for (**A**) hematocrit, (**B**) hemoglobin, and (**C**) mean corpuscular hemoglobin concentration (two-way repeated measures ANOVA; $p > 0.05$; see Results for details). (**D**) Splenic somatic index was not significantly different between control and treatment groups at post-acclimation (Welch's *t*-test; $t = -0.51$; $df = 7.54$; $p = 0.628$). See Tables S4-5 for details on sample size. Individual data points are overlayed with mean ± S.E.M. (control presented in blue, treatment presented in purple). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

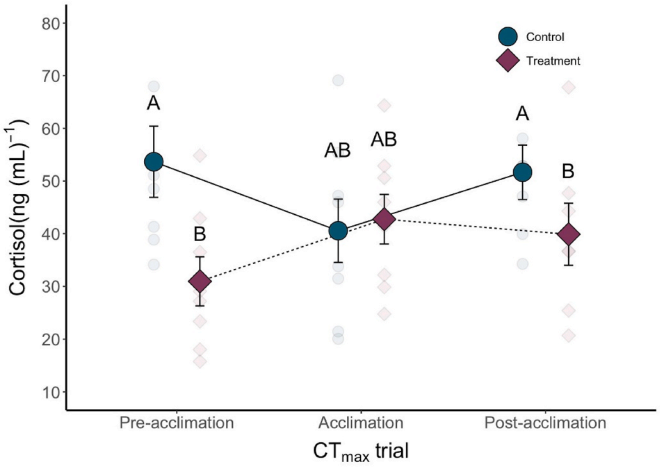

Fig. 6. Plasma cortisol response of subadult white sturgeon maintained at 14°C (control) or sturgeon which underwent a warm acclimation at 20°C and were re-acclimated to 14°C (treatment) immediately after CT_{max} trials. There was a significant interaction between treatment and trial (two-way repeated measures ANOVA; $F_{1.88,20.72} = 3.87$; $p = 0.039$). Letters denote statistically significant differences as detected by subsequent Tukey HSD post-hoc analysis (see Results and Table S1 for details). $n = 7$ for control at pre-acclimation and post-acclimation, and $n = 8$ for control at acclimation. $n = 8$ for treatment at pre-acclimation and acclimation, and $n = 6$ for post-acclimation. Individual data points are overlayed with mean ± S.E.M. (control presented in blue, treatment presented in purple). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

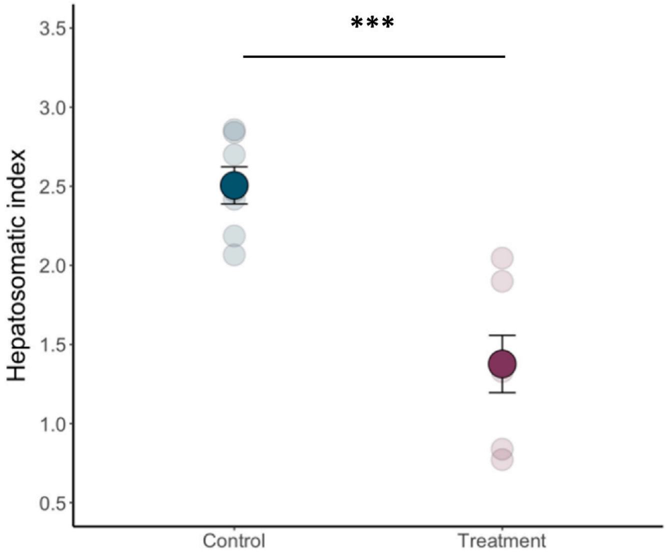

Fig. 7. Hepatosomatic index (HSI) of subadult white sturgeon maintained at 14°C (control) or sturgeon which underwent a warm acclimation at 20°C and were re-acclimated to 14°C (treatment) after post-acclimation CT_{max} trial. HSI was significantly different between control and treatment groups (Student's t-test; $t = 5.23$; $df = 12$; $p < 0.001$). Individual data points ($n = 7$ for each group at post-acclimation) are overlayed with mean ± S.E.M. (control presented in blue, treatment presented in purple). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

thermal plasticity may be particularly advantageous when coping with rapid environmental changes. However, we know little about acclimation potential of later life-stages, often due to the logistical constraints of large-bodied animals. In this study, we characterized how thermal tolerance changes with warm acclimation in 10-year-old subadult white sturgeon, and for the first time, their response after returning to cooler control temperatures post-acclimation. As predicted, sturgeon displayed incredible phenotypic plasticity, adjusting their thermal tolerance in response to their acclimation temperature – increasing in warm acclimation and decreasing when returned to control conditions. Furthermore, we found contrary to our predictions, that acclimation temperature did not impact their stress responses. Surprisingly, acclimation temperature instead had a lasting effect on relative liver size, demonstrating that even after one month of re-acclimating to control conditions, energetic stores had been altered by chronic warm temperatures. These findings suggest that the underlying physiological mechanisms that allow for increased thermal plasticity may have a prolonged recovery period.

4.1. *The effect of acclimation temperature on thermal tolerance*

As predicted, white sturgeon increased CT_{max} after a month-long period of acclimation to 20°C (Fig. 2). This plasticity in upper thermal limits has been previously reported in other fishes and is particularly pronounced in sturgeon species, including green, shortnose, Atlantic and lake sturgeon (Bard and Kieffer, 2019; Bugg et al., 2020; Penny et al., 2023; Rodgers et al., 2019; Zhang and Kieffer, 2014). Yet recent research suggests white sturgeon display some of the highest capacities for thermal acclimation, specifically in early life stages. In fact, white sturgeon yolk-sac larvae exhibit the largest ARR published to date of 1.4 between 14°C and 18°C acclimation temperatures (Earhart et al., 2023); in other words, for every 1°C of acclimation, larvae are increasing their upper thermal limits by 1.4°C. In the present study, 10-year-old white sturgeon exhibited an ARR of 0.52 in response to a +6°C warm acclimation. Though this pales in comparison to their thermally plastic early-life counterparts, white sturgeon here and in general display a greater capacity to increase upper thermal limits in a compensatory manner than previously reported for aquatic ectotherms (Gunderson and Stillman, 2015; Morley et al., 2019). In contrast to trends in acclimation capacity, our data suggest that subadults have higher upper thermal limits than early life stage white sturgeon and are more tolerant to warm temperatures in general. This supports the work of Dahlke et al. (2020), suggesting there exists a "thermal bottleneck" at early life stages, despite greater acclimation capacity. More studies are needed to characterize how thermal tolerance and acclimation capacity change across life stages in white sturgeon, and will be critical for identifying optimal life stage-specific thermal conditions.

We also found that sturgeon lost that increase in thermal tolerance after a month-long period of re-acclimation to 14°C, as predicted (Fig. 2). While there are no significant differences between thermal tolerance at pre-acclimation and post-acclimation for the treatment group, it is interesting to note that CT_{max} post-acclimation is still elevated, indicated by the reduction in ARR to 0.31 in response to the acclimation from 20°C to 14°C. This may suggest white sturgeon have a higher capacity for acclimation to warm temperature, whereas re-acclimation to control temperature after prolonged exposure to sub-optimal temperature may be energetically costly. Instead, we see some marginal conservation of increased thermal tolerance, such that the treatment group have a higher thermal safety margin post-acclimation. Thermal safety margins (the difference between acclimation or environmental temperature and CT_{max}) generally narrows with increasing acclimation temperature (Sandblom et al., 2016), and many species display smaller thermal safety margins with warming (e.g., New Zealand triplefin fish, *Forsterygion lapillum*; McArley et al., 2017). At pre-acclimation temperature, the thermal safety margins for subadult white sturgeon for the control and treatment groups are 15.9°C and 15.8°C, respectively. Control fish retain this thermal safety margin at acclimation (16.3°C) and post-acclimation (16.0°C). For treatment fish, thermal safety margin is reduced during warm acclimation (12.9°C), much like other fish species. However, post-acclimation, these sturgeon have a thermal safety margin of 17.1°C; therefore, prior exposure to warm temperatures may provide a marginal benefit to tolerate future warming. Research is needed to further explore any benefits warm acclimation provides that could lessen the negative impact of increasing temperatures on cold-water fish and even marginally protect threatened species in the face of climate change.

4.2. *Repeatability of CT_{max}*

With our experimental design, we were also able to test the repeatability of CT_{max} tests with white sturgeon for the first time. The ecological relevance of this widely used metric has been questioned (reviewed by Desforges et al., 2023). With this, there is a growing interest in using the repeatability of thermal tolerance to illustrate within-individual trait variation and to infer the heritability of thermal tolerance phenotypes and adaptive capacity of species as a whole (Killen et al., 2016; Morgan et al., 2018). Subadult white sturgeon demonstrated a similar level of repeatability ($R = 0.48$) as compared with previous reports for zebrafish (*Danio rerio*; $R = 0.45$; Morgan et al., 2018), shortnose sturgeon (correlation coefficient = 0.57; Bard and Kieffer, 2019), and brook trout (*Salvelinus fontinalis*; $R = 0.48$; O'Donnell et al., 2020). Interestingly, we found that CT_{max} was highly repeatable between subsequent trials but decreased drastically between the first and third trials (Fig. 4). While we agree that overall, these data demonstrate the robust nature of CT_{max} tests, we believe further research is necessary to fully understand the within-individual variation we found here, particularly regarding the low repeatability found between pre-acclimation and post-acclimation CT_{max}.

4.3. *What physiological mechanisms allow for sturgeon thermal plasticity?*

As we predicted warm acclimation would increase thermal tolerance, we sought to characterize the stress response to acute thermal tolerance tests and understand if any plastic changes occur to deal with this acute stress in a different manner allowing for increased tolerance to high heat. However, in our model species, white sturgeon, we did not observe any significant changes in hematological response (Fig. 5). In some fish, rising temperatures has been linked to reduced aerobic capacity as a result of decreased oxygen binding in hemoglobin (Lund and Tufts, 2003; Zhang and Kieffer, 2014). An active reef fish species (*Caesio cuning*) demonstrated increased splenic Hb concentrations after 4 weeks of warm acclimation, indicating ongoing recovery of RBC production and storage, and altered oxygen transport capacity (Johansen et al., 2021). We expected to observe increased hemoglobin and/or increased hematocrit after acclimation at 20°C to compensate for decreased oxygen binding; however, no change in MCHC or circulating RBCs was noted.

It is possible the similarity in stress responses between warm- and control-acclimated fish is due to the length of the acclimation period. Some short-term acclimation studies have reported that increased temperature prompted a significant increase in cortisol concentration (Madaro et al., 2018; Pottinger and Feuchtmayr, 2020), as did a one-month acclimation combined with the acute thermal stress of CT_{max} (Penny and Pavey, 2021); but here, warm-acclimated fish were no more or less stressed than fish maintained at 14°C (Fig. 6). Instead, control fish demonstrated higher stress from the start. Interestingly, 9 of the 16 fish tested here were used for a pilot study on thermal tolerance in April 2022, and recovered post-CT_{max} for 4 months prior to the start of this study. We first speculated that the higher cortisol concentrations seen in the control group were due to this prior exposure to a CT_{max} trial; however, these fish were randomly split between the control and treatment groups (n = 4 and 5, respectively). Though it did not impact recovery, nor was it impacted by prior exposure or warm acclimation, further research is needed to understand what is driving these differences in inherent stress response to acute thermal stress and if these results are applicable to other species.

Energy requirements typically increase with warmer temperatures (e.g., Sandblom et al., 2016), and we predicted subadult white sturgeon would have mobilized glycogen reserves during acclimation to 20°C resulting in decreased HSI seen in other fishes (Bugg et al., 2020; Penman et al., 2023; Rossi et al., 2017; Stone and Sidell, 1981). However, we were surprised to find the treatment fish had significantly lower HSI (approximately 45% reduction compared to control), even after a month-long recovery at 14°C (Fig. 7). In contrast, zebrafish initially deplete their energetic stores (liver lipid and carbohydrate content) in response to warm temperature, but fully recover after 28 days acclimation (Vergauwen et al., 2010). It is possible the time course for recovery is longer with cooler temperatures, as rainbow trout (*Oncorhynchus mykiss*) demonstrated a markedly slower change in fatty acid composition with a cold acclimation from 20°C to 5°C (10–17 days) compared with a warm acclimation from 5°C to 20°C (2–6 days) (Sellner and Hazel, 1982).

In truth, little is known about the lasting impacts of warm acclimation and what the recovery profile may look like for white sturgeon and other fishes. In cold water fishes, a higher resting metabolic rate may be a consequence of warm acclimation resulting in this continued depletion of energy during recovery, which would invariably have consequences for performance and fitness. In fact, lower HSI may be tied to lower immune function. In lake sturgeon, HSI had strong correlative relationship with time to mortality with infection, where fish with lower relative liver size were more susceptible to endotoxin-driven mortality (Bugg et al., 2023). However, most traits tested here besides HSI were incredibly thermally plastic, and the energetic stores necessary to increase HSI after chronic thermal exposure may simply require a longer re-acclimation. Moreover, other compensatory mechanisms not measured here, like metabolic adjustments, may be occurring simultaneously and offset negative effects of lower energetic stores. Further research is needed to examine the long-term implications of depleted energy storage, to determine the length of time needed for recovery after periods of thermal stress, and to understand how co-occurring and prolonged stressors may impact this recovery.

5. Conclusions

In the age of rapid environmental change, long-lived, endangered white sturgeon are an important model species for understanding how phenotypic plasticity may buffer species from negative impacts of acute and chronic environmental stressors. In this study, we demonstrated the incredible thermal plasticity of subadult white sturgeon in response to environmental temperature changes. This thermal plasticity allows the sturgeon to rapidly acclimate to increases in temperature and suggests they are well-equipped to survive the increasingly frequent heatwaves in British Columbia. Prolonged exposure to warm temperatures did not impact their stress response; however, they exhibited depleted energy stores a month after recovery from warm acclimation. While this physiological change most likely aided their performance in warm temperatures, the long recovery period may hinder their response to subsequent environmental changes. Studies are needed to investigate if other fishes with comparable abilities to increase upper thermal limits demonstrate similar thermal plasticity upon re-acclimation, and if prolonged decreases in HSI is a common response to chronic thermal exposure across species. Importantly, though thermal tolerance seems unaffected with re-acclimation, we do not know how other performance metrics (e.g., resting and active metabolism) may be impacted by this lasting effect of warm acclimation. Future experiments will be necessary to determine the long-term ramifications of prolonged thermal stress and the benefits of thermal plasticity in surviving climate change.

Funding sources

This work was supported by a National Science Foundation (NSF) Postdoctoral Research Fellowship in Biology (#2109765) to AMD, and a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development grant (CRDPJ523640-18) to CJB.

CRediT authorship contribution statement

Theresa A. Weber: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Angelina M. Dichiera:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Colin J. Brauner:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no competing interests that could influence the work reported in this study.

Data availability

Data used in this study are archived in the repository figshare (https://doi.org/10.6084/m9.figshare.24081813.v1).

Acknowledgements

We are grateful for the UBC Zoology Aquatics Facility staff (Pat Tamkee and Eric Lotto), D. Montgomery, G. Le, E. Ghanizadeh-Kazerouni, and J. Hoskins for animal care assistance and systems maintenance. We also thank B. Negrete, Jr and G. Le for their thoughtful advice with experimental assays, K. Flear for assistance determining repeatability, and R. Dhillon for the amazing white sturgeon illustrations. This work was supported by a National Science Foundation (NSF) Postdoctoral Research Fellowship in Biology (#2109765) to AMD, and a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development grant (CRDPJ523640-18) to CJB.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.103807.

References

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F., Chown, S.L., 2013. Heat freezes niche evolution. Ecol. Lett. 16, 1206–1219.

Bard, B., Kieffer, J.D., 2019. The effects of repeat acute thermal stress on the critical thermal maximum (CTmax) and physiology of juvenile shortnose sturgeon (*Acipenser brevirostrum*). Can. J. Zool. 97, 567–572.

Beaman, J.E., White, C.R., Seebacher, F., 2016. Evolution of plasticity: mechanistic link between development and reversible acclimation. Trends Ecol. Evol. 31, 237–249.

Beitinger, T.L., Bennett, W.A., McCauley, R.W., 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ. Biol. Fish. 58, 237–275.

Bennett, J.M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., Araújo, M.B., Algar, A.C., Clusella-Trullas, S., Hawkins, B.A., 2021. The evolution of critical thermal limits of life on Earth. Nat. Commun. 12, 1198.

Bugg, W.S., Yoon, G.R., Schoen, A.N., Laluk, A., Brandt, C., Anderson, W.G., Jeffries, K.M., 2020. Effects of acclimation temperature on the thermal physiology in two geographically distinct populations of lake sturgeon (*Acipenser fulvescens*). Conservation Physiology 8 coaa087-coaa087.

Bugg, W.S., Yoon, G.R., Schoen, A.N., Weinrauch, A.M., Jeffries, K.M., Anderson, W.G., 2023. Elevated temperatures dampen the innate immune capacity of developing lake sturgeon (*Acipenser fulvescens*). J. Exp. Biol. 226, jeb245335.

COSEWIC, 2012. COSEWIC Assessment and Status Report on the White Sturgeon *Acipenser transmontanus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xxvii + 75.

Dahlke, F.T., Wohlrab, S., Butzin, M., Pörtner, H.O., 2020. Thermal bottlenecks in the life cycle define climate vulnerability of fish. Science 369 (6499), 65–70.

Desforges, J.E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K.M., Eliason, E.J., Dressler, T.L., McKenzie, D.J., Bates, A.E., Lawrence, M.J., Fangue, N., 2023. The ecological relevance of critical thermal maxima methodology (CTM) for fishes. J. Fish Bio. 102, 1000–1016.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. USA 105, 6668–6672.

Earhart, M.L., Blanchard, T.S., Morrison, P.R., Strowbridge, N., Penman, R.J., Brauner, C. J., Schulte, P.M., Baker, D.W., 2023. Identification of upper thermal thresholds during development in the endangered Nechako white sturgeon with management implications for a regulated river. Conservation Physiology 11, coad032.

Evans, T.G., Diamond, S.E., Kelly, M.W., 2015. Mechanistic species distribution modelling as a link between physiology and conservation. Conservation Physiology 3, cov056.

Fry, F., 1947. Effects of the environment on animal activity. Publ. Out. Fish. Res. Lab. 55, 1–62.

Fry, F., Hart, J.S., 1948. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. 94, 66–77.

Fulton, T.W., 1904. On the spawning of the cod (*Gadus morrhua L.*) in autumn in the North sea. Sea 6, 6–53.

Gomez Isaza, D.F., Rodgers, E.M., 2022. Exercise training does not affect heat tolerance in Chinook salmon (*Oncorhynchus tshawytscha*). Comp. Biochem. Physiol. Mol. Integr. Physiol. 270, 111229.

Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc. Biol. Sci. 282, 20150401.

Han, D., Huang, S.S., Wang, W.-F., Deng, D.-F., Hung, S.S., 2012. Starvation reduces the heat shock protein responses in white sturgeon larvae. Environ. Biol. Fish. 93, 333–342.

Hildebrand, L., Drauch Schreier, A., Lepla, K., McAdam, S., McLellan, J., Parsley, M.J., Paragamian, V., Young, S., 2016. Status of White Sturgeon (*Acipenser transmontanus* Richardson, 1863) throughout the species range, threats to survival, and prognosis for the future. J. Appl. Ichthyol. 32, 261–312.

IPCC, 2023. In: *Climate Change 2023: Synthesis Report.* Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland, pp. 35–115. https://doi.org/10.59327/IPCC/AR6-9789291691647.

Johansen, J.L., Nadler, L.E., Habary, A., Bowden, A.J., Rummer, J., 2021. Thermal acclimation of tropical coral reef fishes to global heat waves. Elife 10, e59162.

Jutfelt, F., Norin, T., Åsheim, E.R., Rowsey, L.E., Andreassen, A.H., Morgan, R., Clark, T. D., Speers-Roesch, B., 2021. 'Aerobic scope protection'reduces ectotherm growth under warming. Funct. Ecol. 35, 1397–1407.

Kieffer, J.D., Bard, B., 2022. Critical thermal maximum and minimum of juvenile shortnose sturgeon (*Acipenser brevirostrum*) acclimated to 12 and 18° C. J. Appl. Ichthyol. 38, 526–530.

Killen, S.S., Adriaenssens, B., Marras, S., Claireaux, G., Cooke, S.J., 2016. Context dependency of trait repeatability and its relevance for management and conservation of fish populations. Conservation Physiology 4.

Lee, S., Hung, S.S., Fangue, N.A., Haller, L., Verhille, C.E., Zhao, J., Todgham, A.E., 2016. Effects of feed restriction on the upper temperature tolerance and heat shock response in juvenile green and white sturgeon. Comp. Biochem. Physiol. Mol. Integr. Physiol. 198, 87–95.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Package "Emmeans". R package version 4.0-3.

Lund, S., Tufts, B., 2003. The physiological effects of heat stress and the role of heat shock proteins in rainbow trout (*Oncorhynchus f*) red blood cells. Fish Physiol. Biochem. 29, 1–12.

Madaro, A., Folkedal, O., Maiolo, S., Alvanopoulou, M., Olsen, R.E., 2018. Effects of acclimation temperature on cortisol and oxygen consumption in Atlantic salmon (*Salmo salar*) post-smolt exposed to acute stress. Aquaculture 497, 331–335.

McArley, T.J., Hickey, A.J.R., Herbert, N.A., 2017. Chronic warm exposure impairs growth performance and reduces thermal safety margins in the common triplefin fish (*Forsterygion lapillum*). J. Exp. Biol. 220, 3527–3535.

Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305, 994–997.

Morgan, R., Finnøen, M.H., Jensen, H., Pélabon, C., Jutfelt, F., 2020. Low potential for evolutionary rescue from climate change in a tropical fish. Proc. Natl. Acad. Sci. USA 117, 33365–33372.

Morgan, R., Finnøen, M.H., Jutfelt, F., 2018. CTmax is repeatable and doesn't reduce growth in zebrafish. Sci. Rep. 8, 7099.

Morley, S., Peck, L., Sunday, J., Heiser, S., Bates, A., 2019. Physiological acclimation and persistence of ectothermic species under extreme heat events. Global Ecol. Biogeogr. 28, 1018–1037.

Muñoz, N.J., Farrell, A.P., Heath, J.W., Neff, B.D., 2018. Hematocrit is associated with thermal tolerance and modulated by developmental temperature in juvenile Chinook salmon. Physiol. Biochem. Zool. 91 (1), 757–762.

Nash, R.D., Valencia, A.H., Geffen, A.J., 2006. The origin of Fulton's condition factor—setting the record straight. Fisheries 31, 236–238.

Nikinmaa, M., 2011. Red blood cell function. In: Farrell, A.P. (Ed.), Encyclopedia Of Fish Physiology, pp. 879–886.

O'Donnell, M.J., Regish, A.M., McCormick, S.D., Letcher, B.H., 2020. How repeatable is CTmax within individual brook trout over short-and long-time intervals? J. Therm. Biol. 89, 102559.

Oliver, E.C., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L.V., Benthuysen, J.A., Feng, M., Sen Gupta, A., Hobday, A.J., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9, 1–12.

Pearson, M., Stevens, E., 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 9, 39–50.

Penman, R.J., Bugg, W., Rost-Komiya, B., Earhart, M.L., Brauner, C.J., 2023. Slow heating rates increase thermal tolerance and alter mRNA HSP expression in juvenile white sturgeon (*Acipenser transmontanus*). J. Therm. Biol., 103599

Penny, F., Bugg, W.S., Kieffer, J.D., Jeffries, K.M., Pavey, S.A., 2023. Atlantic sturgeon and shortnose sturgeon exhibit highly divergent transcriptomic responses to acute heat stress. Comp. Biochem. Physiol. Genom. Proteonomics 45, 101058.

Penny, F., Pavey, S., 2021. Increased acute thermal tolerance and little change to hematology following acclimation to warm water in juvenile Striped Bass, *Morone saxatilis*. Environ. Biol. Fish. 104, 489–500.

Perry, A.L., Low, P.J., Ellis, J.R., Reynolds, J.D., 2005. Climate change and distribution shifts in marine fishes. Science 308, 1912–1915.

Pottier, P., Burke, S., Zhang, R.Y., Noble, D.W., Schwanz, L.E., Drobniak, S.M., Nakagawa, S., 2022. Developmental plasticity in thermal tolerance: ontogenetic variation, persistence, and future directions. Ecol. Lett. 25, 2245–2268.

Pottinger, T.G., Feuchtmayr, H., 2020. The short-term stress response of three-spined sticklebacks to climate-related stressors: a mesocosm study. Hydrobiologia 847, 3691–3703.

Richter, K., Haslbeck, M., Buchner, J., 2010. The heat shock response: life on the verge of death. Mol. Cell 40, 253–266.

Rodgers, E.M., Todgham, A.E., Connon, R.E., Fangue, N.A., 2019. Stressor interactions in freshwater habitats: effects of cold water exposure and food limitation on early-life growth and upper thermal tolerance in white sturgeon, *Acipenser transmontanus*. Freshw. Biol. 64, 348–358.

Rossi, A., Bacchetta, C., Cazenave, J., 2017. Effect of thermal stress on metabolic and oxidative stress biomarkers of *Hoplosternum littorale* (Teleostei, Callichthyidae). Ecol. Indicat. 79, 361–370.

Sandblom, E., Clark, T.D., Gräns, A., Ekström, A., Brijs, J., Sundström, L.F., Odelström, A., Adill, A., Aho, T., Jutfelt, F., 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nat. Commun. 7, 11447.

Sardella, B.A., Sanmarti, E., Kültz, D., 2008. The acute temperature tolerance of green sturgeon (*Acipenser medirostris*) and the effect of environmental salinity. J. Exp. Zool. Part A: Ecological Genetics and Physiology 309 (8), 477–483.

Seebacher, F., White, C.R., Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat. Clim. Change 5, 61–66.

Sellner, P.A., Hazel, J.R., 1982. Time course of changes in fatty acid composition of gills and liver from rainbow trout (*Salmo gairdneri*) during thermal acclimation. J. Exp. Zool. 221, 159–168.

Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M.S., 2015. *Afex*: Analysis of Factorial Experiments. *R package version 0.13–145*.

Spear, M.C., Kieffer, J.D., 2016. Critical thermal maxima and hematology for juvenile Atlantic (*Acipenser oxyrinchus* Mitchill 1815) and shortnose (*Acipenser brevirostrum* Lesueur, 1818) sturgeons. J. Appl. Ichthyol. 32, 251–257.

Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol. Evol. 8, 1639–1644.

Stone, B.B., Sidell, B.D., 1981. Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. I. Alterations in carbon sources for hepatic energy metabolism. J. Exp. Zool. 218, 371–379.

Strowbridge, N., Northrup, S.L., Earhart, M.L., Blanchard, T.S., Schulte, P.M., 2021. Acute measures of upper thermal and hypoxia tolerance are not reliable predictors of mortality following environmental challenges in rainbow trout (*Oncorhynchus mykiss*). Conservation Physiology 9, coab095.

Sunday, J., Bennett, J.M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A.L., Leiva, F.P., Verberk, W.C., Olalla-Tárraga, M.Á., Morales-Castilla, I., 2019. Thermal tolerance patterns across latitude and elevation. Philosophical Transactions of the Royal Society B 374, 20190036.

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proc. Biol. Sci. 278, 1823–1830.

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2012. Thermal tolerance and the global redistribution of animals. Nat. Clim. Change 2, 686–690.

Vargas-Chacoff, L., Regish, A.M., Weinstock, A., McCormick, S.D., 2018. Effects of elevated temperature on osmoregulation and stress responses in Atlantic salmon *Salmo salar* smolts in fresh water and seawater. J. Fish. Biol. 93 (3), 550–559.

Vergauwen, L., Benoot, D., Blust, R., Knapen, D., 2010. Long-term warm or cold acclimation elicits a specific transcriptional response and affects energy metabolism in zebrafish. Comp. Biochem. Physiol. Mol. Integr. Physiol. 157, 149–157.

Yusishen, M.E., Yoon, G.R., Bugg, W., Jeffries, K.M., Currie, S., Anderson, W.G., 2020. Love thy neighbor: social buffering following exposure to an acute thermal stressor in a gregarious fish, the lake sturgeon (*Acipenser fulvescens*). Comp. Biochem. Physiol. Mol. Integr. Physiol. 243, 110686-110686.

Zhang, Y., Kieffer, J.D., 2014. Critical thermal maximum (CTmax) and hematology of shortnose sturgeons (*Acipenser brevirostrum*) acclimated to three temperatures. Can. J. Zool. 92, 215–221.

Ziegeweid, J.R., Jennings, C.A., Peterson, D.L., 2008. Thermal maxima for juvenile shortnose sturgeon acclimated to different temperatures. Environ. Biol. Fish. 82, 299–307.